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CHRONOLOGICAL TABLE

1956 April	Institute for Virus Research, Kyoto University, was founded with two departments (Pathology and Biophysics).
1956 April	Scientific Lectures for the Public were presented commemorating the opening of the Institute (the successive Memorial Lecture Series have been presented annually hereafter).
1957 April	Department of Biochemistry and Department of Serology and Immunology were established.
1958 April	Department of Prevention and Therapeutics was established.
1958 December	"Advances in Virology", Vol. 1 (in Japanese) was published as collection of the Memorial Lectures (the successive volumes were published annually hereafter until 1960).
1958 December	"Annual Report of the Institute for Virus Research", Vol. 1, was published (the successive volumes have been published annually hereafter).
1959 July	Virus Diagnosis Center was established.
1961 October	The 1st Symposium of the Institute for Virus Research was held under the auspices of the Institute with the nationwide participants. The proceedings of the Symposium were published as the first issue of the new series of "Advances in Virology" in Japanese (the successive Symposia have been held and their proceedings published annually hereafter).
1962 April	Department of Tumor Virus was established.
1962 October	Several staff members were appointed academic staff of the Graduate School of Medicine, and students of the School were first admitted to the Institute.
1962 December	Several staff members were appointed academic staff of the Graduate School of Science, and students of the School were first admitted to the Institute.
1964 April	Virus Diagnosis Center was renamed Virological Diagnosis Center.
1965 September	Construction of the new building for the Institute commenced.
1967 March	Construction of the new building was completed.
1968 April	Department of Genetics was established.
1974 April	Department of Molecular and Cellular Virology was established.
1977 April	Department of Neurological Virus Disease was established as such that Visiting Staff be appointed.
1978 April	Animal Laboratory for Experimental Virus Infection was established.
1981 March	Construction of extension of the main building was completed. Thus the main building now constitutes five floors with a basement occupying the aggregate area of 5,410 ㎡. The major part (ca. 481 ㎡) of the extended area serves for researches

	involving radioisotope labelling and in vitro DNA recombination experiments requiring the P3 facilities.
1986 May	The memorial events for the 30th anniversary of foundation of this Institute were held on May 16-17.
1986 November	Professor Yorio Hinuma was honoured as "Person of Cultural Merits (Bunkakorosha)"
1987 May	Department of Biophysics and Department of Tumor Virology were reorganized to form Department of Viral Oncology which consists of 4 Laboratories.
1988 April	Virological Diagnosis Center was reorganized to become Research Center for Immunodeficiency Virus which consists of Laboratory for AIDS Immunology and Laboratory of Viral Pathogenesis.
1989 April	Department of Biochemistry and Department of Genetics were reorganized to form Department of Genetics and Molecular Biology which consists of 3 Laboratories.
1990 March	Construction of a new building was partly completed.
1990 April	Department of Pathology and Department of Molecular and Cellular Virology were reorganized to form Department of Cell Biology which consists of 3 Laboratories, while Department of Serology and Immunology, Department of Prevention and Therapeutics and Department of Neurological Virus Disease were reorganized to form Department of Biological Responses which consists of 2 laboratories and one for visiting staff.
1992 April	Laboratory of Regulatory Information was established within the Department of Cell Biology to host a visiting professor as well as a research group.
1993 December	Construction of the new building which accommodates three laboratories from this Institute as well as some from the Medical School and the Center for Molecular Biology and Genetics of the University was completed.
1994 October	Construction of a new animal facility with some laboratories was completed.
1998 April	One stuff member was appointed academic staff of the Graduate School of Pharmaceutical Sciences, and students of the school were first admitted to the Institute.
1998 April	Research Center for Immunodeficiency Virus was reorganized to become Research Center for Acquired Immunodeficiency Syndrome.
1998 April	Laboratory of Virus Control in Research Center for Immunodeficiency Virus was established as such that Visiting Stuff be appointed.
1999 April	Several staff members were appointed academic staff of the Graduate School of Biostudies, and students of the school were first admitted to the Institute.
2002 April	The Experimental Research Center for Infected Animals was abolished and the Experimental Research Center for Infectious Diseases was established instead.

2005 April	Research Center for Emerging Virus was established.
2009 Jun	The Institute commenced service as a Joint Usage / Research Center for fusion of advanced technologies and innovative approaches to viral infections and life science.
2010 April	Center for Acquired Immunodeficiency Syndrome Research was reorganized to become Center for Human Retrovirus Research.

ORGANIZATION AND STAFF

(as of December, 2010)

(Numerals in parentheses indicate year of association with the Institute)

Director Masao Matsuoka, M.D., D.Med.Sc.
Deputy Director Yoichi Shinkai, D.Med.Sc.

Professors Emeriti
Yoshimi Kawade, D.Sc. (1956-1988)
Yorio Hinuma, M.D., D.Med.Sc. (1980-1988)
Masao Hanaoka, M.D., D.Med.Sc. (1959-1989)
Mutsuo Imai, D.Sc. (1965-1991)
Takashi Yura, D.Sc. (1960-1993)
Masakazu Hatanaka, M.D., D.Med.Sc. (1980-1995)
Akinori Ishimoto, M.D., D.Med.Sc. (1964-1968, 1978-2002)
Yoshiaki Ito, M.D., D.Med.Sc. (1984-2002)
Masanori Hayami, D.V.M., D.Agr. (1988-2006)
Koreaki Ito, D.Sc. (1971-2007)
Junji Yodoi, M.D., D.Med.Sc. (1989-2010)

Department of Viral Oncology

Laboratory of Gene Analysis
- Professor: Yoshinori Akiyama, D.Sc. (1988)
- Associate Professor: Hiroyuki Sakai, D.Med.Sc. (1996); Hiroyuki Mori, D.Sc. (1996)
- Assistant Professor: Shin-ichi Yanagawa, D.Agr. (1986)

Laboratory of Cell Regulation
- Professor: Masahiko Sugita, M.D., D.Med.Sc. (2004)
- Associate Professor: Isamu Matsunaga, M.D., D.Med.Sc. (2004)
- Assistant Professor: Hirotaka Kuwata, D.D.S., Ph.D. (2010)

Laboratory of Tumor Biogenesis
- Professor: Shin Yonehara, D.Sc. (1994) (concurrent)

Laboratory of Human Tumor Viruses
- Associate Professor: Makoto Hijikata, D.Med.Sc. (1997)

Department of Genetics and Molecular Biology

Laboratory of Molecular Genetics
- Professor: Takashi Fujita, D.Sc. (2005)
- Associate Professor: Hiroki Kato, D.Med.Sc. (2010)

Laboratory of Biochemistry
- Professor: Mutsuhito Ohno, D.Sc. (2001)
- Assistant Professor: Makoto Kitabatake, D.Sc.(2004); Ichiro Taniguchi, D.Sc. (2007)

Laboratory of Genetic Information Analysis
- Associate Professor: Haruo Ohmori, D.Sc. (1979)

Department of Biological Responses

Laboratory of Biological Protection
- Professor: Koichi Ikuta, M.D., D.Med.Sc. (2002)
- Assistant Professor: Masamichi Ueda, D.Sc. (1978); Keiko Takemoto, D.Sc. (1992); Shizue Tani-ichi, D.Health Sc. (2007); Takahiro Hara, D. Bio. (2008)

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Laboratory of Subcellular Biogenesis
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Assistant Professor Shigeru Matsumura, D.Bio. (2008)
Laboratory of Growth Regulation
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Associate Professor Toshiyuki Ohtsuka, M.D., D.Med.Sc. (2000)
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Laboratory of Primate Model
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Assistant Professor Shin-ichiro Narita, D.Sc. (2010)

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Hiroaki Takeuchi
Yusuke Yanagi
Takeshi Noda
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Koichi Morita

Library

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Finance	Yoshifumu Yamasaki (2008)

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Takashi Yura	Dept. Viral Oncol. (Lab. Gene Analy.)
Yoshiyuki Matsuo	Dept. Biol. Resp. (Lab. Inf. Prev.)
Aitor Gonzalez	Dept. Cell Biol. (Lab. Growth Regul.)
Hiromi Shimojo	Dept. Cell Biol. (Lab. Growth Regul.)
Akihiro Isomura	Dept. Cell Biol. (Lab. Growth Regul.)
Shigeki Hoshino	Dept. Cell Biol. (Lab. Signal Transduction.)
Eiji Sato	Dept. Cell Biol. (Lab. Signal Transduction.)
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Yasuteru Sakurai	Res. Cen. AIDS (Lab. Virus Immunol.)

Graduate Students

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Yang Chia-Ming	Exp. Res. Cen. Inf. Dis. (Lab. Mouse Model)
Katsuaki Deguchi	Exp. Res. Cen. Inf. Dis. (Lab. Mouse Model)
Daisuke Muramatsu	Exp. Res. Cen. Inf. Dis. (Lab. Mouse Model)
Mayuko Inoue	Exp. Res. Cen. Inf. Dis. (Lab. Mouse Model)
Yutaro Yamaguchi	Exp. Res. Cen. Inf. Dis. (Lab. Mouse Model)

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Research Activities

LABORATORY OF BIOCHEMISTRY

LABORATORY OF GENE INFORMATION ANALYSIS

DEPARTMENT OF BIOLOGICAL RESPONSES

LABORATORY OF BIOLOGICAL PROTECTION

LABORATORY OF SIGNAL TRANSDUCTION

CENTER FOR HUMAN RETROVIRUS RESEARCH

LABORATORY OF VIRAL PATHOGENESIS

ウイルス研究所 － － － 2010

DEPARTMENT OF VIRAL ONCOLOGY
LABORATORY OF GENE ANALYSIS

I. First Group

The research projects carried out in this group are concerned with post-translational events in the expression of genetic information. Specifically, processes of protein translation, protein translocation across and integration into the membrane, membrane protein proteolysis and extracytoplasmic stress responses are investigated by combined molecular genetic, biochemical and structural approaches.

1) Functions of SecDF, a protein export enhancing membrane component: H. MORI, T. TSUKAZAKI[1], Y. ECHIZEN[1], O. NUREKI[1] and K. ITO[2]

([1]School of Sci., The Univ. Tokyo and [2]Kyoto Sangyo Univ.)

The SecYEG translocon and the SecA ATPase cooperatively facilitate protein export across the bacterial cytoplasmic membrane. Although efficient protein export requires transmembrane proton-motive force as well as SecD-SecF, a pair of membrane integrated Sec factors, their mechanisms of actions remain unknown. We showed that a post-initiation translocation step that can proceed in the absence of ATP requires both SecDF and the proton-motive force, and that the first periplasmic domain (P1) of SecDF interacts with translocating substrates. To obtain a structural basis for its function, we have determined the crystal structure of SecDF from *Thermus thermophilus* at 3.3 Å resolution (see last year's report). The full-length structure revealed a pseudo-symmetrical, 12-helix transmembrane domain, belonging to the RND exporter superfamily. Structural and functional analyses showed that the substrate-binding P1 domain undergoes functionally important conformational changes. In accordance with its similarity with the RND proteins, we found that conserved Asp and Arg residues in the transmembrane SecD/SecF-interface are essential for protein export. Finally, our finding of a Na^+-dependent SecDF paralog in *Vibrio alginolyticus* provided physiological evidence for the cation-coupled translocation mediated by SecDF. Based on these results, we propose that SecDF functions as a dynamic, membrane-integrated chaperone, which is powered by the cation-motive force to facilitate ATP-independent continuation and completion of protein translocation.

2) Toward identification of SecDF nearest neighbors using *in vivo* site-directed photo cross-linking: Y. MACHIDA, Y. AKIYAMA and H. MORI

SecD and SecF form a membrane integrated protein complex that facilitates protein export using PMF generated across the cytoplasmic membrane (see above). Although these proteins are

suggested to form complexes with the SecYEG translocon and YidC, an integral protein involved in membrane protein biogenesis, little is known about the nature of subunit contacts in these complexes. To elucidate how SecD and SecF interact with other proteins *in vivo*, we utilized the *in vivo* site-directed photo crosslinking approach developed by P. Schultz and co-workers (1). Based on the crystal structure of *Thermus thermophilus* SecDF, we constructed eight *E. coli* SecD derivatives containing a pBPA (p-benzoyl-phenylalanine, a photo reactive amino acid analogue) in the membrane boundary regions of transmembrane segment (TM) 2, 3, 5 or 6, which are predicted to locate on the surface of the SecDF complex, and carried out *in vivo* photo-crosslinking experiments. Upon UV-irradiation, specific cross-linked products were detected when pBPA was introduced at positions 580, 584 and 595 in the SecD TM6 region. Now we are trying to identify partner factors of the cross-linked products by immunoblotting using specific antibodies against candidate proteins.

(1) Chin, J.W., Martin, A..B., King, D.S., Wang, L. and Schultz, P.G. (2002) Proc. Natl. Acad. Sci. USA, 99 ,11020-4

3) Membrane targeting of heat shock factor σ^{32} is required for feedback control of heat shock response: T. YURA, H. MORI, K. ITO[1], B. LIM[2], C. GROSS[2] and Y. AKIYAMA ([1]Kyoto Sangyo University, [2]University of California)

Heat shock response is a major homeostatic mechanism for controlling the state of protein folding and degradation in all organisms. Expression of heat shock genes in *E. coli* is under positive control by σ^{32} and negative feedback control (inactivation/degradation of σ^{32}) by chaperones (DnaK/J, GroEL/S) that bind native σ^{32}. σ^{32} is extremely unstable *in vivo* and is degraded by the membrane-localized FtsH protease; whereas chaperones contribute to rapid degradation of σ^{32} *in vivo*, degradation *in vitro* is very slow and not enhanced by chaperones. Although a simple model based on the binding competition between chaperones and RNA polymerase for σ^{32} had long been thought to explain feedback control, our recent work with σ^{32} mutants defective in feedback control revealed unexpected complexity in regulation (1).

In searching for missing factors, we found a novel feedback-resistant mutant with a transposon inserted upstream of the chromosomal *ftsY* gene (encoding SRP receptor) which reduces the FtsY level. Genetic and biochemical analyses of interplay between σ^{32} and the SRP-pathway, mostly done during T.Y.'s stay in UCSF, suggested a novel regulatory pathway for the heat shock response, namely the SRP-dependent targeting of σ^{32} to the membrane. To further extend this line of work, we examined the involvement of N-terminal sequence of σ^{32} in membrane targeting and feedback control of σ^{32}. By using a transposon probe Tn*phoA*, we found that N-terminal 52 amino acids of σ^{32} containing part of the conserved 'region 2.1' acts as a signal for SRP-dependent export of alkaline phosphatase to the periplasm: this export is markedly reduced by several mutations affecting feedback control including the Tn*5* insertion into P*ftsY*, an 'integration-defective' SecY

translocon mutation, and feedback-resistant mutations within region 2.1 of σ^{32} identified previously (1). These results suggest that the same (or overlapping) sequence serves as a signal for membrane targeting of σ^{32} for feedback control during normal growth and upon heat stress to sustain protein homeostasis. We expect that the membrane localization of σ^{32} facilitates inactivation and/or degradation of σ^{32} under excess chaperones and help co-ordinate the cellular responses to changing protein-folding states between cytoplasm and the membrane.

(1) Yura, T., Guisbert, E., Poritz, M., Lu, C.Z., Campbell, E. and Gross, C.A. (2007) Proc. Natl. Acad. Sci. USA, 104, 17638-17643.

4) Roles of the two PDZ domains of RseP, the S2P family intramembrane protease of *E. coli,* in regulation of its protease function: Y. HIZUKURI and Y. AKIYAMA

The *E. coli* σ^E pathway of extracytoplasmic stress response (ESR) is activated through sequential proteolytic cleavages of a membrane-bound anti-σ^E protein, RseA, by membrane proteases DegS and RseP. RseP has tandem, circularly permutated PDZ domains (PDZ-N and PDZ-C) in its periplasmic region. Our recent *in vivo* and *in vitro* studies showed that several mutations in the putative ligand-binding pocket of PDZ-N make RseP capable of cleaving full-length RseA independently of the first cleavage by DegS, suggesting that the PDZ-N domain plays an important role in regulation of the two-step proteolysis of RseA through binding of an as yet unidentified ligand (1). Recently, Li and colleagues proposed a new model based on the crystal structures of PDZ-N/C and the *in vitro* experiments using solubilized proteins, in which cleavage of RseA by RseP is facilitated through direct recognition by PDZ-C of the newly exposed carboxyl-terminal residue of the DegS-cleaved RseA (2). We tested this model by *in vivo* experiments. Li *et al*. have reported that the identity of the newly exposed C-terminal residue Val^{148} of the site-1 cleaved RseA is important for efficient site-2 cleavage, because its substitution with charged or dissimilar amino acid abolished the site-2 cleavage *in vitro*. However, our results showed that a derivative of RseA148 (the DegS-cleaved form of RseA) having any one of the other 19 a.a. residues at its C-terminus (position 148) was efficiently cleaved by RseP *in vivo*. Moreover, although Li *et al*. have reported that a mutation in the putative ligand-binding grooves of the PDZ domains (I215A of PDZ-N or I304A of PDZ-C) almost completely prevented cleavage of RseA *in vitro*, we found that the RseP variants with these mutations cleaved RseA model substrates as efficiently as wild type RseP *in vivo*. These results strongly suggest that recognition of the exposed substrate C-terminal residue by the RseP PDZ domains makes little, if at all, contribution to substrate cleavage by RseP *in vivo*. Furthermore, we constructed a strain carrying the chromosomal *rseP(ΔPDZ-C)* gene encoding an RseP derivative devoid of the PDZ-C domain and found that this mutant exhibited normal σ^E activation in response to overproduction of OmpC, a cue for the σ^E pathway ESR. This result suggests that the RseP PDZ-C domain is not required for regulation of RseP in the OMP-induced stress response. Now we are trying to identify a physiological ligand of

RseP PDZ by using site-directed *in vivo* photo-cross-linking experiment. We have preliminarily detected some cross-linked products.

(1) Inaba, K., Suzuki, M., Maegawa, K. -i., Akiyama, S., and Akiyama, Y. (2008) J. Biol. Chem., 283, 35042-35052.

(2) Li, X., Wang, B., Feng, L., Kang, H., Qi, Y., Wang, J., and Shi, Y. (2009) Proc. Natl. Acad. Sci. USA., 106, 14837-14842.

5) An attempt to identify a substrate motif for *E. coli* rhomboid protease GlpG: K. TERUSHIMA, H. MORI and Y. AKIYAMA

Rhomboid proteases, a family of intramembrane cleaving proteases (I-CLiPs) that are thought to hydrolyze substrate membrane proteins within the membrane, are involved in a wide range of biological events including EGFR signaling, host cell invasion by protozoan parasites and bacterial quorum sensing. We have been studying *E. coli* GlpG, a member of rhomboid proteases. As a model rhomboid enzyme, GlpG has been extensively studied biochemically and structurally, but its physiological substrate and cellular function remain unknown. Recently, Strisovsky *et al*. (1) showed that several residues (at positions P4, P1 and P2') surrounding the cleavage site are crucial for cleavage of rhomboid substrates and proposed the "consensus substrate motif" recognized by rhomboid proteases. However, the proposed motif does not completely fit with our previous data obtained form the analysis of model substrate cleavage by GlpG, suggesting that the motif recognized by GlpG somewhat deviates from the proposed consensus. To elucidate the GlpG recognition motif, we started systematic mutational analysis against the cleavage site region in a model substrate of GlpG. Our preliminary results suggest that position P1' is additionally important for substrate cleavage by GlpG. We will further extend the analysis to elucidate the GlpG-specific substrate motif, which would help us to identify a physiological substrate and cellular roles of GlpG.

(1) Strisovsky, K., Sharpe, H.J, and Freeman M. (2009) Mol. Cell, 36, 1048-1059.

6) X-ray crystal structural analysis of membrane bound ATP-dependent protease FtsH: R. SUNO, A, ABE, Y. AKIYAMA, S. IWATA[1] and M. YOSHIDA[2] ([1]Department of medicine, Kyoto University, [2]the Chemical Resources Laboratory, Tokyo Institute of Technology)

ATP-dependent proteases are involved in various cellular processes including cell division, cell differentiation, signal transduction, and stress response. FtsH degrades not only misassembled subunits of membrane protein complexes for their quality control but also some short-lived cytosolic regulatory proteins for cellular regulation. FtsH comprises an N-terminal transmembrane segment and a C-terminal cytosolic region, which consists of AAA^+ (ATPases associated with diverse cellular activities) and protease domains. Previously, we successfully crystallized and

determined a soluble region of FtsH (sFtsH) containing ADP from *T. thermophilus* at 3.9 Å resolution. In the hexameric structure, a substrate polypeptide can reach the active protease catalytic sites through a tunnel leading from AAA^+ domain of the adjacent subunit, but not from the central axial region. This raises a possibility of direct delivery of a polypeptide through this tunnel. Recently, we succeeded in crystallizing sFtsH with several kinds of ATP analogues to understand the molecular mechanism of FtsH in detail. The diffraction data were collected at the beamline BL41 XU at Spring-8 at 100 K. These crystals diffracted at least 3.5 Å resolution. Now, we are analyzing these data to determine new crystal structures of sFtsH bound several ATP analogues.

7) Biochemical analysis of the substrate-translocating mechanism of ATP-dependent Protease FtsH: R. SUNO, M, SHIMOYAMA[1], A. ABE, N. SHIMODATE[1], Y. AKIYAMA and M. YOSHIDA[1] ([1]the Chemical Resources Laboratory, Tokyo Institute of Technology)

The structural analysis also suggested that several mobile regions play an important role in the operating mode of FtsH. Based on the structural information, it is conceivable that a β-hairpin and a lid-helix, which presumably form the tunnel, are involved in translocating the polypeptide. The lid-helix covering the protease catalytic site can kink at the position of the highly conserved Gly448. Substitution of this residue by other amino acids resulted in the decrease of ATPase activity and the complete loss of ATP-dependent protease activity. It was considered that these mutations impaired the flexibility of the lid-helix, leading to a more rigid FtsH with impaired functionality.

II. Second Group

1) Analysis of Molecular Mechanism Underlying Keratin-Associated Protein 13-Induced Activation of Canonical Wnt Signaling Pathway: S. YANAGAWA

Wnt is known to promote recruitment of Axin by Low-density lipoprotein receptor-related protein 6 (LRP6), a co-receptor for Wnt that leads to accumulation of β-catenin and activation of Wnt pathway. I found that Keratin associated protein (Krtap) 13, a cysteine-rich cytoplasmic protein binds to LRP6. Surprisingly, Krtap13 overexpression markedly stimulates Wnt signaling, suggesting that Krtap13 activates Wnt signaling by mimicking some aspects of normal Wnt signal transduction. Actually, Krtap13 overexpression induced accumulation of β-catenin. In addition, I found that Krtap13 binds to both LRP6 and Dvl and that overexpression of Krtap13 promotes Dvl-aggregates formation. Wnt treatment is known to induce plasma membrane–associated LRP6

aggregates (LRP6 signalosomes), which contain Dvl and Axin. Thus, a possible molecular mechanism underlying Krtap13-induced activation of Wnt signaling is to induce co-clustering of LRP6 and Dvls, thereby mimic function of LRP6 signalosomes.

To analyze effect of ectopic expression of Krtap13 in vivo, I am trying to establish transgenic mouse lines that express Krtap13 in a tissue specific manner. For this purpose, 4 lines of transgenic mouse carrying a trans-gene consisting of CAG-promoter, loxp-polyA-loxp cassette, and 3XFLAG-tagged human Krtap13 cDNA were established. By crossing these Krtap13-transgenic mice with another transgenic mice that express Cre in a tissue-specific way, I am expecting to analyze effect of tissue specific overexpression of Krtap13 in mice.

2) Growth control by estrogen in the HPV positive cells: A. SATSUKA, N. KAJITANI and H. SAKAI

The estrogen has been reported to be involved in several types of cancer development. Recent reports suggested that the estrogen and its nuclear receptor promoted cervical cancer. However the effects of estrogen in the HPV replication remains to be understood. We examined the regulation of the HPV gene expression by the estrogen treatment.

The estrogen treatment repressed the activity of HPV early promoter in HPV positive cell line and the cell growth. Estrogen might promote the cervical cancer progression, but the shut down of its pathway seems to be essential to maintain the cencer cell proliferation.

3) Analysis of CAF formation mechanism using HPV positive cells: A. SATSUKA, N. KAJITANI and H. SAKAI

In many reports, the importance of the interaction between the cancer stem cells and the microenvironments has been indicated. In the previous studies, it was suggested that HPV E6, E7, c-Myc, and H-ras were the key factors for the establishment of the cancer stem cell in the cervical cancer. These factors might alter the microenvironment to be favorable for cancer development. To examine the effect of the cancer cells in fostering the cancer-associated fibroblasts (CAFs), HPV-positive cancer cells, SiHa, HeLa and Caski, were applied to the organotypic raft culture, and the effects on the fibroblasts were analyzed by gene-expression profiling. The expressions of CD44 and α-SMA were used as the markers for the CAF induction. In another experiment, the fibroblasts expressing an oncogene, *myc*, *src*, or *ras* were used as the transformed fibroblasts, and normal HFKs or HeLa cells were overlaid on these cells. The effect of TGFβ produced by CAFs on the EMT of normal and HPV-positive keratinocytes was also examined. These inter-cellular communications might be important for the progression of the cervical cancer.

4) Identification of Novel Function of Human Papillomavirus E4: N. KAJITANI, A. SATSUKA and H. SAKAI

HPV infection begins in the basal cells of the epithelium, and as these cells divide, differentiate, and migrate toward the surface of the epithelium, the virus is able to complete its life cycle. The viral life cycle depends on the differentiation of the epithelium, but how the life cycle is controlled is not well understood. It is interesting that viral oncoproteins cause the increase of cellular proliferation and/or transformation, but terminally cellular differentiation of epithelium is required for completion of the viral life cycle.

The expression of E4 occurs in the upper layers of the HPV-infected epithelium, coordinating with the onset of viral genome amplification and the expression of viral late genes. It is known that E4 disrupts the keratin networks. It is also known that E4 induces G_2/M cell cycle arrest. But it is yet to be known well about the details of E4. To investigate novel functions of E4, we performed yeast two-hybrid assays and got several candidate proteins as which interacts with E4.We carry on the analysis about the interactions between the each candidates and E4 in vitro or in vivo. In the future, we will ascertain the function of E4 and its involvement in the viral life cycle.

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DEPARTMENT OF VIRAL ONCOLOGY
LABORATORY OF GENE ANALYSIS

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Hizukuri, Y., Kojima, S. and Homma, M. Disulphide cross-linking between the stator and the bearing components in the bacterial flagellar motor. J. Biochem. 148, 309–318, 2010.

鈴木守、稲葉謙次、前川憲一、秋山修志、伊藤維昭、秋山芳展：大腸菌膜内切断プロテアーゼ RseP の PDZ ドメインの構造解析. 第 23 回日本放射光学会年会、姫路、２０１０年１月６日―９日

Tsukazaki, T., Mori, H., Ito, K., and Nureki, O.: Structural Analysis of Bacterial Sec Translocon machinery. Gordon Research Conferences on Protein Transport Across Cell Membranes, Galveston, U.S.A, March 7-12, 2010

鈴木守、稲葉謙次、前川憲一、秋山修志、伊藤維昭、秋山芳展：大腸菌膜内切断プロテアーゼ RseP の X 線結晶構造解析. 第 27 回 PF シンポジウム、つくば、２０１０年３月９日―１０日

照島功祐、遠藤政幸、勝田陽介、日高久美、杉山弘 ： RNA ポリメラーゼの DNA ナノ構造上

での 1 分子観測.　日本化学会第９０春季年会、大阪、２０１０年３月２６日―３月２９日

森　博幸、塚崎智也、越前友香、濡木理、伊藤維昭：バクテリアのタンパク質膜透過装置の構造と機能.　日本細菌学会ワークショップ「細菌のタンパク質分泌系」、横浜、２０１０年３月２７日―２９日

森　博幸：タンパク質膜透過装置補助因子 SecDF の構造と機能　遺伝研研究会「単細胞システムの細胞構築と増殖機構の研究」、三島、２０１０年 ３月３０日―３１日

Akiyama, Y.: New function of RseP, the *E. coli* S2P family protease. Gordon Research Conference on Proteolytic enzymes & their inhibitors, Lucca, Italy, May 2-7, 2010.

檜作 洋平、秋山芳展：表層ストレス応答に関わる膜内プロテアーゼ RseP の PDZ ドメインによる活性制御機構解析. 第７回２１世紀大腸菌研究会、熊本、２０１０年６月３日―４日

塚崎智也、森 博幸、越前由香、石谷隆一郎、深井周也、田中剛史、Perederina, A., Vassylyev, D. G.、河野俊之、伊藤維昭、濡木理：Sec トランスロコンと共に機能する SecDF 膜タンパク質の構造　第 10 回蛋白質科学会年会、札幌、２０１０年６月１６日―１８日

檜作 洋平、秋山芳展：細菌表層ストレス応答に関与する膜内プロテアーゼ RseP の PDZ ドメインによる活性制御機構解析. 特定領域研究「タンパク質の社会」若手ワークショップ、福岡、２０１０年７月１日―３日

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Mori, H., Tsukazaki, T., Nureki, O., Ito, K. and Akiyama, Y.: A functionally important intramolecular interaction in SecA: involvement of hydrophobic amino acids in motif IV and anti-parallel beta sheet in translocase activation. The 3rd International Symposium on Protein Community, Nara, September 13-16, 2010

Tsukazaki, T., Mori, H., Echizen, Y., Ishitani, R., Fukai, S., Tanaka, T., Perederina, A., Vassylyev, D. G., Kohno, T., Ito, K. and Nureki, O.: Crystal structure of SecDF, a Sec translocon-associated membrane protein. The 3rd International Symposium on Protein Community, Nara, September 13-16, 2010

Yura, T., Lim, B., Gross, C., Ito, K., Mori, H. and Akiyama, Y.: SRP-dependent targeting of sigma 32 to the membrane: A critical step for the chaperone-mediated feedback control in bacterial heat shock response. The 3rd International Symposium on Protein Community, Nara, September 13-16, 2010

Ito, K., Chiba, C., Akiyama, Y. and Abo, T.: Visualizing dynamic "nascentome" of the cell. The 3rd International Symposium on Protein Community, Nara, September 13-16, 2010

Suno, R., Shimoyama, M., Shimodate, N., Abe, A., Akiyama, Y., and Yoshida, M.: The role of the mobile regions in the ATP-dependent protease activity of FtsH. The 3rd International Symposium on Protein Community, Nara, September 13-16, 2010

Hizukuri, Y. and Akiyama, Y.: Roles of the two PDZ domains of RseP, the S2P family intramembrane protease of *Escherichia coli,* in regulation of its protease function. The 3rd International Symposium on Protein Community, Nara, Japan, September 13-16, 2010

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寿野良二、下立夏香、下山真和、阿部明子、秋山芳展、吉田賢右：膜結合型 ATP 依存性プロテアーゼ FtsH の構造と機能. 日本生体エネルギー研究会、大阪、２０１０年１１月１８日―２０日

斎藤 啓、千葉志信、松尾英一、西村 紀、伊藤維昭、秋山芳展 : Fate of signal peptides in bacteria: post liberation cleavage by S2P protease. 第３３回日本分子生物学会大会・第８３会日本生化学会大会合同大会　ワークショップ「膜タンパク質の構造・機能から見るオルガネラの進化」、神戸、２０１０年１２月７日―１０日

Mori H., Tsukazaki, T., Akiyama Y., Nureki O. and Ito K.: Structure, function and evolution of bacterial protein translocation machinery. 第３３回日本分子生物学会年会・第８３回日本生化学会大会合同大会　ワークショップ「進化からみたタンパク質社会」、神戸、２０１０年 １２月７日―１０日

檜作 洋平、秋山芳展 : 大腸菌表層ストレス応答に関わる S2P 膜内プロテアーゼ RseP の PDZ ドメイン機能の解析. 第３３回日本分子生物学会年会・第８３回日本生化学会大会合同大会、神戸、２０１０年１２月７日―１０日

II. Second Group

Satsuka, A., Yoshida, S., Kajitani, N., Nakamura, H., and Sakai, H.: A novel human papillomavirus type18 replicon and its application in screening the anti-viral effects of cytokines. Cancer Sci. 101: 536-542, 2010.

柳川伸一：Analysis of molecular mechanism underlying Keratin-associated protein13-induced activation of canonical Wnt signaling pathway. 第３３回日本分子生物学会年会、神戸、２０１０年１２月７日―１０日

佐塚文乃、梶谷直子、酒井博幸：エストロジェン刺激による HPV 陽性細胞の増殖制御. 第５８回日本ウイルス学会学術集会、徳島、２０１０年１１月７日―９日

佐塚文乃、梶谷直子、酒井博幸：Mechanisms of the HPV-induced transformation. 第６９回日

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佐塚文乃、梶谷直子、酒井博幸 : Novel human papillomavirus type 18 replicon and its application in screening the antiviral effects of cytokines. 第３３会日本分子生物学会年会・第８３回日本生化学大会合同大会、神戸、２０１０年１２月７日―１０日

DEPARTMENT OF VIRAL ONCOLOGY
LABORATORY OF CELL REGULATION

The universe of antigens recognized by T lymphocytes has recently been expanded to include not only protein antigens but also lipid antigens. Unlike conventional MHC molecules that present protein-derived peptide antigens, molecules of the human group 1 CD1 family (CD1a, CD1b, CD1c) mediate presentation of lipid antigens to specific T lymphocytes. By taking lipid chemical and immunological approaches and by developing appropriate animal models (human CD1 transgenic mice, guinea pigs, and non-human primates), we aim at determining how CD1 has evolved to function critically in host defense against microbial infection and cancer. Further, inappropriate immune responses to lipids may result in induction of allergy and autoimmune diseases. These critical aspects of the newly recognized lipid-specific immunity have now been addressed in our laboratory.

1) Reconstitution of the human CD1a expression and function in mice: C. KOBAYASHI, T. SHIINA[1] and M. SUGITA ([1]Tokai Univ.)

Mice and rats are useful animals for many immunological studies, but important exceptions exist. These animals have deleted genes for group 1 CD1 family, and thus, lack the lipid recognition system that is comparable to that in humans. Given the necessity of appropriate small animal models for monitoring CD1-mediated immune responses *in vivo*, we attempted to develop two distinct, but complementary, animal systems; namely, guinea pigs and CD1 transgenic mice. We have recently found that guinea pigs have evolved the CD1 system equivalent to that in humans, capable of mounting the CD1-restricted T cell response to mycobacterial lipids. On the other hand, the paucity of critical reagents often hampers detailed analysis of CD1-mediated immune responses in guinea pigs. As an alternative animal model, we generated CD1a transgenic mice carrying the human *CD1A* genome. The expression of CD1a molecules in these mice was detected exclusively in epidermal Langerhans cells and immature thymocytes, thus precisely representing CD1a distribution in humans. By establishing CD1a transgenic mice that lack the expression of either GM-CSF, sulfatide, or MHC class II, we are now analyzing how CD1a expression is regulated and what T cell subsets may react to CD1a molecules.

2) Identification of a mycobacteria-derived glycolipid that delayed-type hypersensitivity targets: T. KOMORI, I. MATSUNAGA, Y. HATTORI, H. KUWATA, H. HARASHIMA[1] and M. SUGITA ([1]Hokkaido Univ.)

In guinea pig models of infection with bacillus Calmette-Guerin (BCG), an attenuated

vaccine strain of *Mycobacterirum bovis*, we obtained evidence for the delayed-type hypersensitivity (DTH) directed against a glycolipid antigen. Pathogenic mycobacteria produce glucose monomycolate (GMM), a glucosylated species of mycolic acids, by utilizing host-derived glucose as a substrate for mycolyltransferases. The host CD1b-based immunity detects GMM and mounts potent Th1-type T cell responses. Given that Th1 cytokines, such as interferon-γ and TNF-α, are critical for host defense against mycobacterial infection, GMM is now considered as a good candidate of lipid-based vaccines against tuberculosis and related diseases. This possibility has been addressed in monkeys by setting up a collaboration with Prof. Igarashi of the Institute.

3) Lipid biology of dormant mycobacteria: T. URAKAWA, I. MATSUNAGA, N. FUJIWARA and M. SUGITA

Control of latent tuberculosis, or infection with dormant mycobacteria, is one of the most critical challenges for global health. Having established an experimental model of dormant mycobacteria, we now detect lipid biosynthesis that occurs preferentially in dormant mycobacteria. Candidate glycolipids detected preferentially in dormant mycobacteria have been identified, and their interaction with the host innate and acquired immunity is being assessed.

4) Lipid immunity in AIDS: D. MORITA, M. HORIIKE[1], T. IGARASHI[1] and M. SUGITA ([1]Laboratory of Primate Model, IVR)

By taking advantage of IVR's superb research environments and close collaboration with Prof. Igarashi's laboratory, this newly launched project addresses how CD1-dependent immunity functions in host defense against retrovirus infection. We have finished delineating the CD1 system in monkeys, highlighting not only expected similarities but also unexpected differences between humans and monkeys. We have now set out to analyze CD1-dependent immunity in SIV-infected monkeys and have identified an array of virus-derived lipidic molecules that the host immunity is able to recognize specifically.

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LABORATORY OF CELL REGULATION

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U.S.A., July 13-15, 2010.

森田大輔、堀池麻理子、三浦智行、森直樹、五十嵐樹彦、杉田昌彦：リポペプチド：エイズ免疫の新たな標的分子　第 13 回京都免疫ワークショップ学術集会　大阪　2010 年 2 月 13 日

杉田昌彦：ミコール酸含有糖脂質の生合成と免疫認識～抗酸菌バイオロジーの新たな世界～　第 85 回日本結核病学術集会　京都　　2010 年 5 月 20-21 日

杉田昌彦：結核菌脂質を標的とした新しい免疫応答　第 53 回日本感染症学会中日本地方会学術集会　京都　2010 年 11 月 12-13 日

松永勇、小森崇矢、藤原永年、杉田昌彦：*Mycobacterium avium* の血清型非特異的糖ペプチド脂質に対する抗体産生　第 33 回日本分子生物学会年会・第 83 回日本生化学会大会　合同大会　神戸　2010 年 12 月 7-10 日

DEPARTMENT OF VIRAL ONCOLOGY
LABORATORY OF TUMOR BIOGENESIS

Apoptosis, or programmed cell death, plays an important role in many biological processes, including embryogenesis, development of immune system, maintenance of tissue homeostasis, and elimination of virus-infected and tumor cells. We found cell surface Fas antigen (Fas), which can directly mediate apoptosis-inducing signals into cells by stimulation with agonistic anti-Fas mAbs or Fas ligand. Our main research project is to understand the intracellular signal transduction mechanism of cell death including apoptosis and caspase-independent novel types of cell death, and the biological significance/physiological role of cell death and cell death-regulating molecules. Investigations of molecular mechanisms and physiological roles of cell death are important for a better understanding of mammalian immune system, embryogenesis and tumorigenesis.

1) Identification of functional regions defining different activity in caspase-3 and caspase-7 within cells: N. NAKATSUMI and S. YONEHARA

Caspases are central to apoptosis, and the principal executioner caspases, caspase-3 and -7, were reported to be similar in activity, primary structure, and three-dimensional structure. Here, we identified different activity in caspase-3 and -7 within cells and examined the relationship between their structure and function using human cells expressing almost equal amounts of exogenous caspase-3, caspase-7 and/or chimeric constructs after down-regulation of endogenous caspase-3 and -7 expression. Caspase-3 (produced in human cells) showed much stronger cleaving activity than caspase-7 against a low molecular weight substrate in vitro dependent on four specific amino acid regions. Within cells, however, an additional three regions were required for caspase-3 to exert much stronger protease activity than caspase-7 against cellular substrates. Three of the former four regions and the latter three regions were shown to form two different three-dimensional structures that were located at the interface of the homodimer of procaspase-7 on opposite sides. In addition, procaspase-3 and -7 revealed specific homodimer-forming activity within cells dependent on five amino acid regions, which were included in the regions critical to the cleaving activity within cells. Thus, human caspase-3 and -7 exhibit differences in protease activity, specific homodimer-forming activity, and three-dimensional structural features, all of which are closely interrelated.

2) Analysis of interaction between FLASH and ARS2 by alanine scanning mutagenesis and its role in cell cycle progression: T. HAMAUCHI, M. KIRIYAMA and S. YONEHARA

FLASH (FLICE/caspase-8-associated huge protein/CASP8AP2) was identified by yeast two-hybrid screening using two tandemly repeated death-effector domains of procaspase-8 as a bait in our laboratory. Recent studies indicated that FLASH plays an important role in cell cycle progression, and expression of histone. In our laboratory, it was shown that 1) FLASH-down-regulated cells are arrested in S phase, 2) FLASH is associated with arsenite resistance protein 2 (ARS2) through its central region composed of 13 amino acids (FARB region), and 3) interaction of FLASH with ARS2 is involved in S phase progression. ARS2 was originally identified as a gene product conferring resistibility to arsenite in an arsenite-hypersensitive cell line, and ARS2-down-regulated cells was indicated to cause growth retardation. Here, by alanine scanning mutagenesis, we identified 3 critical glutamic acid residues in FARB that are necessary to bind to ARS2. Then we generated FLASH mutants where the important glutamic acid residues are replaced by alanine. The mutants of FLASH can neither interact with ARS2 nor sufficiently rescue the growth of endogenous FLASH-down-regulated cells. Thus, interaction of FLASH with ARS2 was indicated to be important in cell growth and cell cycle progression. Moreover, we found that down-regulation of ARS2 expression induces down-regulation of FLASH expression. However, expression of exogenous FLASH in ARS2-down-regulated cells cannot rescue the cell growth retardation. Collectively, we concluded that down-regulation of ARS2 expression induces cell growth retardation in a FLASH independent manner.

3) Establishment of inducible gene expression and knockdown systems in mouse embryonic stem cells to analyze the functions of various genes in differentiation: M. SOMEDA and S. YONEHARA

Embryonic stem (ES) cells, which are derived from the inner cell mass of blastocyst, have the pluripotency to differentiate into various cell types, including neuron. Recent studies on neuronal differentiation of ES cells have suggested that analysis of *in vitro* generation of neural cells from ES cells is a powerful tool to examine neuronal development. To understand gene functions during neuronal differentiation, we established inducible gene expression and knockdown system in mouse ES cells. This system makes exogenous genes or shRNAs available to express in a timely fashion during whole neuronal differentiation. ES clones stably expressing a mutated hormone-binding domain of mouse estrogen receptor Mer-Cre-Mer chimera molecule (MerCreMer) was infected with a lentiviral vector, Lex-puro/EGFP, which carries a puromycin resistant gene (*puro*^{r}) flanked on both sides by a lox site and an EGFP gene. Because of the presence poly(A) site connecting to the *puro*^{r} gene, EGFP cannot express until the *puro*^{r}-poly(A) is deleted by the functions Cre. MerCreMer, which usually remains cytoplasm, translocates into the nucleus and exert its enzymatic activity after treatment with the synthetic ligand to Mer, 4-hydroxytamoxyfen (4-OHT). We also utilized inducible expression system of shRNAs under control of the Tet-On

system. All the ES cell lines we established were shown to be able to differentiate into neural cells by various methods used in *in vitro* neuronal differentiation. In Fas-mediated apoptosis, the death-inducing signaling complex (DISC), composed of death receptor Fas, adaptor protein FADD and caspase-8, mediates the extrinsic pathway inducing apoptosis. Recent studies, however, have indicated that these molecules have non-apoptotic functions in both embryonic development and maintenance of living body. In this study, we investigated functions of these apoptosis-related genes during neuronal differentiation using the inducible gene expression and knockdown systems in mouse ES cells.

4) An Essential role of the Wnt signals in both self renewal and differentiation of mouse ES cells: A. MURAKAMI

Wnt signaling pathways were reported to play an important role in the process of differentiation at the gastrulation stage during embryogenesis. We have been studying a role of the Wnt signals in the differentiation process using mouse ES cells. The ES cells could be induced to differentiated cells under several culture conditions. In case of the ES cells, however, increased Wnt signals rather inhibit differentiation and maintain "stemness" of the cells.

Among the Wnt family members, we have detected Wnt3 and Wnt8a expression in ES cells. Both are assessed as a signaling molecule that stimulates the Wnt canonical pathway. Nevertheless, Knock down of each expression by introducing the shRNA and overexpression of each molecule revealed that Wnt3 and Wnt8a are involved in the opposite processes in ES cells. We are now analyzing what is the difference between signaling pathways mediated by the Wnt3 and Wnt8a, and how they could play such a different role in the differentiation process of ES cells.

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DEPARTMENT OF VIRAL ONCOLOGY
LABORATORY OF HUMAN TUMOR VIRUSES

1) The hepatitis C virus particle requires a disulfide-bonded dimer of core protein : Y. KUSHIMA, T. WAKITA and M. HIJIKATA

Hepatitis C virus (HCV) core protein forms the nucleocapsid of the HCV particle. Although many functions of core protein have been reported, how the HCV particle is assembled is not well understood. Here we show that the nucleocapsid-like particle (capsid) of HCV is composed of a disulfide-bonded dimer of core (dbd-core). Mutational analysis revealed that the cysteine residue at amino-acid position 128 (Cys128) of core, a highly conserved residue among all reported isolates, is responsible for dbd-core formation. Amino-acid substitution at Cys128 resulted in significant reductions in infectious particle production and loss of dbd-core formation. Additionally, the Cys128 mutant core showed a dominant-negative effect in terms of HCV particle production. These results suggest that this disulfide bond is critical for the HCV virion. We also showed that the sensitivity of the dbd-core of HCV capsid against proteinase K but not to trypsin, suggesting that this capsid is built up of a tightly packed structure of the core with its N-terminal arginine-rich region of the core inside and C-terminal hydrophobic region outside.

2) IRF7 dependent IFN-α response in the early phase of the viral infected hepatocytes: Y. QI, H. H. ALY, C. TSUTSUI , T. FUJITA and M. HIJIKATA

The transcription factors, IRF3, IRF7 and NF-κB are known to play crucial roles in innate immune system of the cells. These factors cooperate to induce type I interferon genes after activation by viral infection. Our previous research indicated that IRF7 plays a more important role in the suppression of HCV infection in HuS-E/2 cells, immortalized human hepatocytes, than IRF3. We also observed that IRF7 is constitutively produced in human primary hepatocytes without virus infection, although the expression of IRF7 gene is known to occur by IFN-β induced by activated IRF3 in many cells. These suggested that there is a human hepatocyte-specific innate immune system. So we further analyzed the response of the genes related with innate immune system against viral infection. We found that IFN-α gene expression was induced in the early phase of the virus infection (3 hours post-infection) in both primary hepatocytes and HuS-E/2 cells. It came before induction of IFN-β gene. The suppression of IFN-α1 gene induction in the early phase was observed in HuS-E/2 cells expressing the dominant negative form of IRF7, suggesting that this early induction of IFN-α gene by viral infection is IRF7 dependent.

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DEPARTMENT OF GENETICS AND MOLECULAR BIOLOGY
LABORATORY OF MOLECULAR GENETICS

1) Ser386 phosphorylation of transcription factor IRF-3 induces dimerization and association with CBP/p300 without overall conformational change: K. TAKAHASI, M. HORIUCHI, K. FUJII, S. NAKAMURA, N. N. NODA, M. YONEYAMA, T. FUJITA and F. INAGAKI

The transcription factor IRF-3 is activated by microbial invasions and produces a variety of cytokines including type-I interferon. Upon microbial infection, IRF-3 is phosphorylated at its C-terminal regulatory domain, then oligomerized, translocated into the nucleus, and here it binds to CBP/ p300. Although a number of studies have been reported investigating the activation mechanism of IRF-3, there are a number of unresolved issues, especially on the phosphorylation sites, the oligomerization process and the binding mechanism with CBP/ p300. In this report, the phosphorylated IRF-3 regulatory domain (IRF-3 RD) was prepared using the kinase IKK-i, and the active form of phosphorylated IRF-3 RD was identified. The paper also reports the crystal structure of the active form of the phosphorylated IRF-3 RD. Furthermore, the phosphorylation of Ser386 was found to be essential for its dimerization and binding with CBP/ p300 using mutational analysis and mass spectrometry. Thus, we conclude that the phosphorylation of Ser386 is essential for activation of IRF-3.

2) Virus-Infection or 59ppp-RNA Activates Antiviral Signal through Redistribution of IPS-1 Mediated by MFN1: K. ONOGUCHI, K. ONOMOTO, S. TAKAMATSU, M. JOGI, A. TAKEMURA, S. MORIMOTO, I. JULKUNEN, H. NAMIKI, M. YONEYAMA and T. FUJITA

In virus-infected cells, RIG-I-like receptor (RLR) recognizes cytoplasmic viral RNA and triggers innate immune responses including production of type I and III interferon (IFN) and the subsequent expression of IFN-inducible genes. Interferon-b promoter stimulator 1 (IPS-1, also known as MAVS, VISA and Cardif) is a downstream molecule of RLR and is expressed on the outer membrane of mitochondria. While it is known that the location of IPS-1 is essential to its function, its underlying mechanism is unknown. Our aim in this study was to delineate the function of mitochondria so as to identify more precisely its role in innate immunity. In doing so we discovered that viral infection as well as transfection with 5'ppp-RNA resulted in the redistribution of IPS-1 to form speckle-like aggregates in cells. We further found that Mitofusin 1 (MFN1), a key regulator of mitochondrial fusion and a protein associated with IPS-1 on the outer membrane of mitochondria, positively regulates RLR-mediated innate antiviral responses. Conversely, specific

knockdown of MFN1 abrogates both the virus-induced redistribution of IPS-1 and IFN production. Our study suggests that mitochondria participate in the segregation of IPS-1 through their fusion processes.

3) LGP2 is a positive regulator of RIG-I- and MDA5-mediated antiviral responses: T. SATOH, H. KATO, Y. KUMAGAI, S. SATO, K. MATSUSHIT, T. TUJIMURA, T. FUJITA, S, AKIRA and O. TAKEUCHIT

RNA virus infection is recognized by retinoic acid-inducible gene (RIG)-I-like receptors (RLRs), RIG-I, and melanoma differentiation-associated gene 5 (MDA5) in the cytoplasm. RLRs are comprised of N-terminal caspase-recruitment domains (CARDs) and a DExD/H-box helicase domain. The third member of the RLR family, LGP2, lacks any CARDs and was originally identified as a negative regulator of RLR signaling. In the present study, we generated mice lacking LGP2 and found that LGP2 was required for RIG-I- and MDA5-mediated antiviral responses. In particular, LGP2 was essential for type I IFN production in response to picornaviridae infection. Overexpression of the CARDs from RIG-I and MDA5 in Lgp2(-/-) fibroblasts activated the IFN-beta promoter, suggesting that LGP2 acts upstream of RIG-I and MDA5. We further examined the role of the LGP2 helicase domain by generating mice harboring a point mutation of Lys-30 to Ala (Lgp2 (K30A/K30A)) that abrogated the LGP2 ATPase activity. Lgp2 (K30A/K30A) dendritic cells showed impaired IFN-beta productions in response to various RNA viruses to extents similar to those of Lgp2(-/-) cells. Lgp2(-/-) and Lgp2 (K30A/K30A) mice were highly susceptible to encephalomyocarditis virus infection. Nevertheless, LGP2 and its ATPase activity were dispensable for the responses to synthetic RNA ligands for MDA5 and RIG-I. Taken together, the present data suggest that LGP2 facilitates viral RNA recognition by RIG-I and MDA5 through its ATPase domain.

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DEPARTMENT OF GENETICS AND MOLECULAR BIOLOGY
LABORATORY OF BIOCHEMISTRY

In eukaryotic cells, many genes are separated by introns into multiple exons that should be joined together. In addition, the cell itself is separated by the nuclear envelope into two major compartments, the nucleus and the cytoplasm. These two types of separations necessitate specific gene expression mechanisms such as RNA splicing and nuclear transport. Prof. Mutsuhito OHNO's laboratory is studying various aspects of eukarysotic gene expression with great emphasis on "RNA" as a key molecule. In addition, Assistant Prof. Kitabatake's subgroup is focusing on quality control mechanisms of eukaryotic ribosome particles.

1) RNA distribution in the cell:

1-1) Identity elements used in mRNA export

Different RNA species, such as tRNAs, U snRNAs, mRNAs and rRNAs, utilize distinct export pathways, i.e., distinct sets of export factors. Accumulating evidence shows that the pathway of RNA export can influence the fate of a given RNA in the cytoplasm, indicating the biological importance of the choice of RNA export pathway. This means that the cellular export machinery must be able to discriminate distinct RNA species, and therefore each RNA species should have identifying features that specify its export pathway ("identity elements"). We are mainly focusing on mRNAs and performing a systematic search for identity elements used in export of mRNAs. To this end, we make various chimeric RNAs between mRNA and U1 snRNA, and look for RNA features that make the chimeric RNAs behave like an mRNA rather than a U snRNA in nuclear export process. We also look for the trans-acting factors that recognize the identity elements to elucidate the mechanisms of RNA export pathway choice.

1-2) Molecular mechanisms for nuclear retention of intron-containing mRNA precursors

Intron-containing pre-mRNAs are normally retained in the nucleus until they are spliced to produce mature mRNAs that are exported to the cytoplasm. The nuclear retention of pre-mRNAs is essential for proper gene expression. It secures pre-mRNAs to be efficiently spliced since splicing mainly occurs in the nucleus. It also secures pre-mRNAs not to be translated since translation of pre-mRNAs would possibly produce toxic abnormal proteins for the cell. However, the nuclear retention mechanisms of pre-mRNAs are not well understood, especially in vertebrates. We are trying to understand such mechanisms.

2) rRNA quality control mechanisms:

How the eukaryotic cells deal with non-functional RNA molecules that were either mutated or damaged? We are searcing for novel RNA quality control mechanisms in mammalian and yeast cells by mainly focusing on ribosomal RNAs.

Quality control mechanisms operate in various steps of ribosomal biogenesis to ensure the production of functional ribosome particles. It was previously reported that mature ribosome particles containing nonfunctional mutant rRNAs are also recognized and selectively removed by a cellular quality control system (nonfunctional rRNA decay; NRD). Here, we show that the NRD of 25S rRNA requires a ubiquitin E3 ligase component Rtt101p and its associated protein Mms1p, previously identified as factors involved in DNA repair. We revealed that a group of proteins associated with nonfunctional ribosome particles are ubiquitinated in a Rtt101-Mms1-dependent manner. 25S NRD was disrupted when ubiquitination was inhibited by the overexpression of modified ubiquitin molecules, demonstrating a direct role for ubiquitin in this pathway. These results uncovered an unexpected connection between DNA repair and the quality control of rRNAs. Our findings support a model in which responses to DNA and rRNA damages are triggerd by a common ubiquitin ligase complex, during genotoxic stress harmful to both molecules.

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DEPARTEMENT OF GENETICS AND MOLECULAR BIOLOGY
LABORATORY OF GENE INFORMATION ANALYSIS

1) Overlapping of human REV7- and MAD2-binding motif sequences: T. HANAFUSA, T.HABU[1], J. TOMIDA[1], E. OHASHI[2], Y. MURAKUMO[3] and H. OHMORI ([1]Kyoto University Radiation Biology Center, [2]Kyushu University, [3]Nagoya University)

Polζ, a DNA polymerase specialized for trans-lesion DNA synthesis (TLS), contains at least, two subunits, the REV3 catalytic subunit (consisted of 3130 amino acids) and the REV7 accessory subunit (211 aa). The human REV7 (hREV7) protein is known to interact with hREV3, hREV1 (another TLS protein) and some other proteins such as ADAM9 (a disintegrin and metalloprotease) and ELK-1 (an Ets-like transcriptional factor). hREV7 is alternatively termed hMAD2L2, because its primary sequence shows 26% identity to that of hMAD2. Because hMAD2 plays crucial roles in spindle assembly checkpoint (SAC) *via* interactions with hMAD1 or hCDC20, hMAD2L2/REV7 was to interact with hCDH1, an hCDC20 homologue, as hMAD2 does with hCDC20. As a step to examine whether hREV7/MAD2L2 is involved in both TLS and SAC, we investigated the molecular basis for the interactions of hREV7/MAD2L2 and hMAD2 with their binding partners. Our results revealed that a short sequence of hREV3 (1877-ILKPLMSPP-1885, designated minimum core sequence, MCS in short) was necessary and sufficient for interaction with hREV7, although the presence of several amino acid residues C-terminal to MCS enhanced the hREV7-interaction. Surprisingly, hMAD2 also bound to the MCS in hREV3, while hMAD2 did not bind to a similar sequence in ADAM9 or ELK-1, and hREV7 did not bind to the hMAD2-binding sequence in hMAD1 or hCDC20. While we could detect intracellular interaction between a hREV3 fragment carrying the 1759-2004 sequence and the endogenous hREV7 protein, we could not detect such an interaction between the hREV3 fragment and an over-expressed hMAD2. We infer that the hREV3 sequence surrounding MCS may confer an inhibitory effect on the hREV3-hMAD2 interaction. Furthermore, by yeast two-hybrid assay, we could not detect any interaction between hREV7 and hCDH1, under the conditions where the interaction between hMAD2 and hCDC20 was detected. Thus, we conclude that while hREV7 and hMAD2 have similar recognition sequences, each of them functions separately for TLS and SAC, respectively.

2) Intracellular interaction between REV7 and REV3 in DT40 cells: K. TAKENAKA[1], H. OHMORI and Y. MIKI[1] ([1]Tokyo Medical and Dental University)

We have shown that hREV7 and hMAD2 bind to a 9-aa sequence within hREV3,

1877-ILKPLMSPP-1885, and that amino-acid substitutions in the hREV3 MCS conferred different effects on interactions with hREV7 or hMAD2. For example, I1877A or L1878A substitution in the hREV3 MCS completely abolished the interaction with hMAD2, but either substitution by itself showed no or little effect on the interaction with hREV7 while the I1877A/L1878A double substitution abolished the hREV7-interaction. On the other hand, P1880F substitution abolished the hREV7-interaction, but it fully retained the hMAD2-interaction. Taking advantage of these *in vitro* results, we wished to examine effects of such amino-acid substitutions on intracellular interaction between hREV3 and hREV7 or hMAD2. Since we know it very difficult to detect the intact hREV3 even when over-expressed, we used a truncated form of hREV3 with a FLAG-tag at the N-terminus, FLAG-hREV3(1759-2004), and introduced I1877A or P1880F substitution into the construct. When FLAG-hREV3(1759-2004) carrying the wild-type, I1877A or P1880F mutant sequence was expressed in HEK293 cells, we could detected interaction of the wild-type and I1877A mutant, but not for P1880F mutant, with the endogenous hREV7. To further examine the above mutations for interaction with REV7 *in vivo*, we decided to use DT40, a chicken pre-B cell line that is suited for gene manipulation. The chicken REV7 shows 96% sequence identity with the human REV7 and the chicken REV3 possesses the sequence identical to hREV3 MCS at the identical position. We successfully introduced I1877A or P1880F mutation into the genomic sequence of the DT40 *REV3* gene. Characterizations of such mutant cells should provide more insights into the REV3-REV7 interaction *in vivo*.

3) Interaction between REV3 and REV7 in *S. pombe*: T. HANAFUSA, J. TERUNUMA[1], M. UCHIYAMA[1], F. HANAOKA[1] and H. OHMORI ([1]Gakushuin University)

Our results showed that human REV7 and MAD2, having 23% sequence identity in their primary structures, bind to the same short sequence within hREV3. This indicates that, while the similarity between the entire sequences of hREV7 and hMAD2 is not so high, they share a conserved structure crucial for recognizing a short motif sequence. We then examined if there is any overlapping between REV7- and MAD2-binding motif sequences in other organisms. For this purpose, we studied on REV3-REV7 interaction in *Schizosaccharomyces pombe* (Sp). Using yeast two0hybrid assay, we found that SpREV7 bound to the 517-535 region (517-SFVYKQQPPSTDDLYGTMK-535) of SpREV3 (consisted of 1480 amino acids), which contains a sequence (underlined) with a similarity to the hREV3 MCS. However, SpREV7 bound very weakly to the 9-aa MCS-like sequence and required the downstream sequence for exhibiting a stronger binding. SpMAD2 also bound to the 517-SFVYKQQPP-525 sequence, but its binding was significantly reduced when the upstream sequence was attached to it, as in 513-SQHESFVYKQQPP-525. Furthermore, SpMAD2 bound to the hREV3 MCS, while SpREV7 did not bind to it. Thus, our results indicate that not only in humans but also in yeasts, both REV7

and MAD2 recognize a short motif sequence present within REV3, while their interactions with the MCS-like sequence are differently affected by the surrounding sequences. It should be also noted that the similarity between SpMAD2 and hMAD2 (47% identity) is higher that that between SpREV7 and hREV7 (38% identity). We are now trying to introduce some mutations into the genomic sequence of the *S. pombe REV3* gene and examine intracellular interactions of altered SpREV3 proteins with SpREV7, because SpREV3 is much shorter than hREV3. We'll examine phenotypes of SpREV3 mutants that have completely lost the interaction with SpREV7.

LIST OF PUBLICATIONS
DEPARTEMENT OF GENETICS AND MOLECULAR BIOLOGY
LABORATORY OF GENE INFORMATION ANALYSIS

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J. Akagi, K. Suzuki, E. Ohashi, H. Ohmori and F. Hanaoka：Translesion DNA polymerase Polk acts as an alternative TLS polymerase upon UV-induced DNA lesions in mouse cells、第 32 回日本分子生物学会年会 第８３回日本生化学会大会　合同大会、神戸、１２月 7～１０日、２０１０

DEPARTMENT OF BIOLOGICAL RESPONSES
LABORATORY OF BIOLOGICAL PROTECTION

Our laboratory has made two major achievements. First, we have found that fetal and adult hematopoietic stem cells have different developmental potential to differentiate into lymphocytes. Second, we have demonstrated that interleukin-7 (IL-7) controls DNA recombination of lymphocyte antigen receptor genes by changing chromatin structure. Both of them are related with fundamental questions in medicine and biology.

Based on these findings, we are now pursuing research on development and regulation of the immune system, focusing on the following questions: (1) control mechanism of lymphocyte antigen receptor genes by IL-7; (2) regulation of immune response by IL-7 receptor (IL-7R) expression; and (3) distribution and function of IL-7-producing cells in lymphoid organs.

1) Accessibility control of TCR Vγ region by STAT5: S. TANI-ICHI and K. IKUTA

The signal of the IL-7R and STAT5 plays an essential role in γδ T cell development by inducing V-J recombination in the T cell receptor (TCR) γ locus. Previously, we have shown that STAT5 binds to the Jγ promoters and controls chromatin accessibility by histone acetylation. However, little is known on control mechanism of Vγ region by the IL-7R. To elucidate the regulation by STAT5, we first analyzed the chromatin status of Vγ region in primary thymocytes. The levels of histone H3 acetylation are high at Vγ5, HsA element and Vγ2 in $Rag2^{-/-}$ thymocytes but low in IL-7Rα-deficient early thymocytes, suggesting that IL-7R signaling controls the accessibility of the Vγ region. In addition, high levels of histone H3 acetylation and germline transcription were induced at Vγ5 and HsA by cytokine and STAT5 in cytokine-dependent Ba/F3 and other hematopoietic cell lines. Importantly, the chromatin accessibility of Vγ5 gene is increased by cytokine signal. Furthermore, STAT5 was not recruited to a non-canonical STAT-binding motif in the endogenous chromatin of the Vγ5 promoter by cytokine stimulation in vivo, while STAT5 binds to a consensus motif in the HsA element. In accordance with this result, STAT5 does not directly activate the Vγ5 promoter by reporter assay. These results suggest that while STAT5 directly binds to HsA element and induces its histone acetylation, STAT5 indirectly activates the Vγ5 promoter. Thus, this study implies a potential role of STAT5 in accessibility control of the Vγ region, especially at Vγ5 and HsA.

2) Pre-TCR signal silences the TCRγ locus by inhibiting the recruitment of STAT5 and Runx to transcriptional enhancers: S. TANI-ICHI and K. IKUTA

The mouse TCRγ locus is controlled by transcription factors STAT5 and Runx. While the TCRγ locus is frequently rearranged, its transcription is repressed in αβ T cells. This phenomenon, known as TCRγ silencing, depends on pre-TCR-induced proliferation of thymocytes. The molecular basis for the TCRγ silencing, however, is largely unknown. We showed that pre-TCR signal reduces the transcription and histone acetylation of the TCRγ locus irrespective of V-J rearrangements. We also demonstrated that Runx is recruited to the enhancer elements of the TCRγ locus, Eγ and HsA, mainly at $CD4^-CD8^-$ double negative stage and that its binding is decreased at later stages. Importantly, anti-CD3 Ab treatment decreases the levels of IL-7R expression, STAT5 phosphorylation, and recruitment of STAT5 and Runx to Eγ and HsA elements in RAG2-deficient thymocytes, suggesting that pre-TCR signal inhibits the binding of STAT5 and Runx to the enhancer elements. Furthermore, we observed that introduction of STAT5 or Runx expression vector induces the transcription of TCRγ genes in a DP cell line, DPK. Finally, we showed that the transcription of TCRγ genes is induced in αβ T cells of Runx3 transgenic mice, suggesting that Runx3 has potential to counteract the TCRγ silencing in αβ T cells in vivo. Thus, our results demonstrate that pre-TCR signal inactivates the TCRγ enhancers by inhibiting the recruitment of STAT5 and Runx and imply that this might be an important step for the TCRγ silencing in αβ T cells.

3) STAT5 controls the rearrangement of TCR Jγ gene segments through STAT-binding motifs in the Jγ promoters: K. WAGATSUMA, B. LIANG, S. TANI-ICHI and K. IKUTA

We previously showed that STAT5 activated by IL-7 binds to STAT motifs in Jγ promoters and increases histone acetylation, germline transcription and chromatin accessibility. However, it remains unclear whether the STAT motifs in the Jγ promoters play a critical role in the rearrangements of the TCRγ locus *in vivo*. To address this issue, we generated two kinds of Jγ1 promoter mutant mice. One of them carries mutations in STAT motifs in the Jγ1 promoter (Jγ1P-Stat-mut), and the other has deleted the Jγ1 promoter including the STAT motifs (ΔJγ1P). Flow cytometric analysis showed that $Vγ2^+$ and $Vγ5^+$ T cells of γ1 cluster were severely decreased in the thymus and the small intestine of these mutant mice. In addition, dendritic epidermal T cells exclusively expressing Vγ3 were also reduced in these mice. In contrast, γδ T cells expressing Vγ1.1 of γ4 cluster were unchanged. Furthermore, Vγ–Jγ rearrangements were substantially impaired in the γ1 cluster of these mice, while the rearrangements of other clusters were unchanged. These results demonstrate that recruitment of STAT5 to the STAT motifs in the Jγ1 promoter is essential for rearrangements of the TCR γ1 cluster in vivo, and support the idea that STAT motifs controls local accessibility of the Jγ gene segments.

4) IL-7R controls differentiation of CD8 T cells and maintenance of peripheral T cells: S. TANI-ICHI, A. ABE and K. IKUTA

The IL-7R is essential for differentiation and survival of T cells. We previously showed that IL-7Rα-deficient mice have severely reduced numbers of αβ T cells and completely lack γδ T cells. However, the role of the IL-7R was not precisely determined in late stages of T cell development, because IL-7Rα-deficient mice have profound detrimental effects on early thymocytes. To address this question, we established IL-7Rα-floxed mice and crossed with CD4-Cre transgenic mice. In thymus, total cell numbers of CD4-Cre x IL-7R$\alpha^{flox/flox}$ mice were similar to control mice. Whereas differentiation of $CD4^-CD8^-$, $CD4^+CD8^+$, CD4 single positive (SP) cells and γδ T cells were not affected, the numbers of mature CD8 SP cells were markedly reduced in CD4-Cre x IL-7R$\alpha^{flox/flox}$ thymus. In addition, the development of NKT cells and regulatory T cells were partially impaired in the thymus of CD4-Cre x IL-7R$\alpha^{flox/flox}$ mice. In periphery, although CD4-Cre x IL-7R$\alpha^{flox/flox}$ mice have comparable numbers of lymph nodes and Peyer's patches to control mice, there were a selective loss of CD4 and CD8 T cells and a selective gain of γδ T cells. These data demonstrate that the IL-7R is essential for differentiation of CD8 T cells, NKT cells and regulatory T cells in thymus and maintenance of naive CD4 and CD8 T cells in periphery.

5) Distribution of IL-7-expressing cells in lymphoid tissues: T. HARA, S. SHITARA, G. CUI, S. TANI-ICHI and K. IKUTA

IL-7 is an essential cytokine for lymphocyte development and survival produced by mesenchymal and epithelial cells in lymphoid organs. However, little is known about the precise nature and distribution of IL-7-expressing cells in vivo. To address this question, we established IL-7-GFP knock-in mice. We found that the majority of thymic epithelial cells (TEC) express GFP in the cortex and medulla. A large number of cortical TEC express GFP at high levels, while most medullary TEC express GFP at low levels. Their expression levels decrease gradually with aging. In the lymph node paracortex, fibroblastic reticular cells (FRC) express GFP at intermediate levels. In addition, we detected high levels of GFP expression in lymphatic endothelial cells of lymph nodes, intestines, and skin. In the spleen, FRC scattered in the white pulp express GFP at low levels. Moreover, we found intermediate levels of GFP expression in the stromal cells lining the marginal zone and surrounding central arterioles. In the bone marrow, some $VCAM\text{-}1^+$ stromal cells express GFP at high levels. In the colon, some epithelial cells express high levels of GFP. After induction of acute colitis with DSS, GFP expression was elevated in the intestinal epithelial cells. Thus, the IL-7-GFP knock-in mouse reveals unreported types of IL-7-expressing cells and provides a powerful tool to analyze the IL-7-niche in the lymphoid organs under physiological and pathological conditions.

6) Local function of IL-7 in vivo: T. HARA, B. LIANG, S. SHITARA, K. WAGATSUMA, S. TANI-ICHI and K. IKUTA

IL-7 is an essential cytokine for lymphocyte development and survival produced by epithelial and mesenchymal cells. However, little is known about the local function of IL-7 produced by each cell type in vivo. To address this question, we established IL-7-floxed mice and crossed with FoxN1-Cre Tg mice to obtain the conditional knockout (cKO) mice deficient in IL-7 production from TEC. FoxN1-Cre x IL-$7^{flox/flox}$ mice showed 15-fold reduced numbers of thymocytes compared with control mice. In addition, γδ T and NKT cells are similarly reduced. Interestingly, the reduction and phenotype are less severe than IL-$7^{-/-}$ mice (50-fold reduction), suggesting the possibility that IL-7 produced by mesenchymal cells might play a minor role. In the spleen, the numbers of T cells are partially restored in the cKO mice, indicating homeostatic expansion in the periphery. Therefore, these results suggest that IL-7 produced from TEC plays a major role in proliferation and survival of thymocytes. Interestingly, γδ intraepithelial lymphocytes (IEL) of the small intestine were severely reduced in FoxN1-Cre x IL-$7^{flox/flox}$ mice but not in villin-Cre x IL-$7^{flox/flox}$ mice expressing Cre in intestinal epithelial cells. This result implies the thymic origin of γδ IEL. Next, we crossed the IL7-floxed mice with albumin (Alb)-Cre Tg mice to obtain the cKO mice deficient in IL-7 production from hepatocytes. Alb-Cre x IL-$7^{flox/flox}$ mice showed slightly reduced numbers of NKT cells in adult liver. In addition, B cell development is partially impaired in late fetal and neonatal liver of the cKO mice. These results suggest that IL-7 produced by hepatocyte plays a role in NKT cell maintenance and B cell development in the liver. Thus, this study revealed unknown functions of IL-7 produced from different cells in vivo.

7) ELISA kit system for detecting calreticulin in urine: M. UEDA

Calreticulin (CRT) is the protein that was found in bladder urothelial carcinoma cells. The amount of CRT in urine from patients of bladder urothelial carcinoma was more than that from other urogenital patients. These data were obtained from the immunoblot analyses with PVDF membrane (Kageyama et al. Clin Chem 2004). For diagnosis of urogenital cancer, we tried to construct the assay kit system by ELISA method with HRP. Five independently obtained monoclonal antibodies were used. The sensitivity of these systems was not efficient, and the effective assay kit system for CRT could not be obtained. The other monoclonal antibodies will be produced for this kit system.

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LABORATORY OF BIOLOGICAL PROTECTION

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DEPARTMENT OF BIOLOGICAL RESPONESE
LABORATORY OF INFECTION AND PREVENTION

The research projects carried out in this group are studies on thioredoxin and thioredoxin-related proteins including thioredoxin binding protein-2 (TBP-2)/thioredoxin interacting protein (Txnip)/ Vitamin D3 up-regulated protein 1 (VDUP1) and transmembrane thioredoxin-related protein (TMX). Molecular biology of these proteins is investigated, especially focusing on their important medical and biological aspects such as cancer suppression, as well as the regulation of metabolism and inflammation.

1) Thioredoxin-binding protein-2 (TBP-2/VDUP1/TXNIP) regulates T-cell sensitivity to glucocorticoid during HTLV-I-induced transformation: Z. CHEN, DA. LOPEZ-RAMOS, E. YOSHIHARA, Y. MAEDA, H. MASUTANI, K. SUGIE, M. MAEDA and J. YODOI

Although glucocorticoid (GC) is widely used for treating hematopoietic malignancies including adult T-cell leukemia (ATL), the mechanism by which leukemic cells become resistant to GC in the clinical course remains unclear. Using a series of T-cell lines infected with human T lymphotropic virus type-I (HTLV-I), the causative virus of ATL, we have dissected the transformation from interleukin (IL)-2-dependent to -independent growth stage. The transformation associates the loss of thioredoxin-binding protein-2 (TBP-2), a tumor suppressor and regulator of lipid metabolism. Here we show that TBP-2 is responsible for GC-induced apoptosis in ATL cells. In the IL-2-dependent stage, dexamethasone induced TBP-2 expression and apoptosis, both of which were blocked by GC receptor (GR) antagonist RU486. Knockdown of TBP-2 consistently reduced the amount of GC-induced apoptosis. In IL-2-independent stage, however, expression of GR and TBP-2 was suppressed and GC failed to induce apoptosis. Forced expression of GR led the cells to mild sensitivity to GC, which was also accomplished by treatment with suberoylanilide hydroxamic acid, a TBP-2 inducer. A transfection experiment showed that TBP-2 expression induced apoptosis in IL-2-independent ATL cells. Thus, TBP-2 is likely to be one of the key molecules for GC-induced apoptosis and a potential target for treating the advanced stage of ATL.

2) Differential roles of Annexin A1 (ANXA1/lipocortin-1/lipomodulin) and thioredoxin binding protein-2 (TBP-2/VDUP1/TXNIP) in glucocorticoid signaling of HTLV-I-transformed T cells: Z. CHEN, E. YOSHIHARA, A. SON, Y. MATSUO, H. MASUTANI, K. SUGIE, M. MAEDA and J. YODOI

Glucocorticoid (GC) is widely used for therapeutic purposes in immunological and

hematological disorders. Annexin A1 (ANXA1/lipocortin-1/lipomodulin), a GC-inducible molecule, was regarded as a vital anti-inflammatory mediator of GC. Thioredoxin binding protein-2 (TBP-2/VDUP1/TXNIP), a regulator of redox reactions, cell growth and lipid metabolism, was also reportedly induced by GC. HTLV-I infected T cells undergo the transition from the IL-2 dependent to IL-2 independent growth during the long-term culture in vitro. We found that these T cells responded to GC with growth arrest and apoptosis in the IL-2 dependent growth stage, whereas they failed to respond to GC after their growth had shifted into the IL-2 independent stage. Here we employed these T cell lines and studied the roles of ANXA1 and TBP-2 in mediating GC-induced apoptosis. In GC-sensitive T cells, ANXA1 expression was negligible and unaffected by GC treatment, whereas TBP-2 was expressed and induced by GC treatment. In GC-resistant T cells, however, ANXA1 was highly expressed regardless of GC treatment and promoted cellular proliferation. In contrast, TBP-2 expression was lost and could not mediate the GC-induced apoptosis. In conclusion, these results suggest that TBP-2, but not ANXA1, is directly involved in the switching of GC sensitivity and GC resistance in HTLV-I infected T cell lines, whereas ANXA1 may be a biomarker indicative of the advanced stage of the transformation.

3) Thioredoxin binding protein-2 mediates metabolic adaptation in response to lipopolysaccharide in vivo: S. OKA, W. LIU, E. YOSHIHARA, MK. AHSAN, DA. RAMOS, A. SON, H. OKUYAMA, L. ZHANG, H. MASUTANI, H. NAKAMURA and J. YODOI

Endotoxin triggers a reorganization of the energy metabolic pathway, including the promotion of fatty acid utilization to adapt to a high energy demand during endotoxemia. However, the factors responsible for the metabolic adaptation and characteristic pathologies resulting from defective utilization fatty acids during endotoxin response have not been fully clarified. The thioredoxin binding protein-2 (TBP-2) knockout (TBP-2) mouse is an animal model of fatty acid oxidation disorder. The aim of this study was to determine whether and how TBP-2 is involved in metabolic regulation in a lipopolysaccharide (LPS)-induced endotoxemia model in mice. TBP-2 and wild control mice were intraperitoneally injected with LPS. Mortality, serum levels of markers of hepatorenal injuries, cytokines, insulin, glucose and lipid derivatives, and the hepatic signaling pathway regulating gluconeogenesis were investigated. Following the administration of LPS, TBP-2 mice showed a predisposition for death without any significant elevation of inflammatory cytokines, compared to the wild mice. LPS-challenged TBP-2 mice showed fat deposition in the liver and kidney, organ injuries, glycogen depletion, and elevation of serum lipid derivatives such as free fatty acids, triglyceride and cholesterol. Hyperinsulinemia and hypoglycemia were observed in TBP-2 mice after LPS injection. Death due to the LPS administration was prevented by supplementation of glucose. Phosphorylation of Akt and FoxO1, an inhibitory pathway of

gluconeogenesis in the liver of LPS-challenged TBP-2 mice was demonstrated, suggesting the enhancement of insulin signaling. TBP-2 is involved in metabolic control during LPS-induced endotoxemia. After the LPS challenge, TBP-2 mice showed several characteristic aspects, such as hepatorenal injuries, and dysregulation of the lipid and glucose metabolisms. Furthermore, hypoglycemia promoted by hyperinsulinemia may be a critical risk factor for mortality in circumstances in which fatty acid utilization is impaired during endotoxemia.

4) Disruption of TBP-2 ameliorates insulin sensitivity and secretion without affecting obesity: E. YOSHIHARA, S. FUJIMOTO, N. INAGAKI, K. OKAWA, S. MASAKI, J. YODOI and H. MASUTANI

Type 2 diabetes mellitus (T2DM) is characterized by defects in both insulin sensitivity and glucose-stimulated insulin secretion (GSIS) and is often accompanied by obesity. In this study, we show that disruption of thioredoxin binding protein-2 (TBP-2, also called Txnip) in obese mice (ob/ob) dramatically improves hyperglycaemia and glucose intolerance, without affecting obesity or adipocytokine concentrations. TBP-2-deficient ob/ob mice exhibited enhanced insulin sensitivity with activated insulin receptor substrate-1/Akt signalling in skeletal muscle and GSIS in islets compared with ob/ob mice. The elevation of uncoupling protein-2 (UCP-2) expression in ob/ob islets was downregulated by TBP-2 deficiency. TBP-2 overexpression suppressed glucose-induced adenosine triphosphate production, Ca(2+) influx and GSIS. In β-cells, TBP-2 enhanced the expression level and transcriptional activity of UCP-2 by recruitment of peroxisome proliferator-activated receptor-γ co-activator-1α to the UCP-2 promoter. Thus, TBP-2 is a key regulatory molecule of both insulin sensitivity and GSIS in diabetes, raising the possibility that inhibition of TBP-2 may be a novel therapeutic approach for T2DM.

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DEPARTMENT OF BIOLOGICAL RESPONESE
LABORATORY OF INFECTION AND PREVENTION

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DEPARTMENT OF CELL BIOLOGY
LABORATORY OF SUBCELLULAR BIOGENESIS

1) Genome-wide screening of the kinases required for the control of cell division axis: **S. MATSUMURA, M. HAMASAKI, M. EBISUYA[1], T. YAMAMOTO[2], E. NISHIDA[3] and F. TOYOSHIMA** ([1]ICDO, Career-Path Promotion Unit, Kyoto University, [2]iCeMS, Kyoto University, [3]Graduate School of Biostudies, Kyoto University)

Oriented cell division, which is determined by the axis of a mitotic spindle, plays an essential role in morphogenesis, asymmetric cell division and stem cell self-renewal. There is increasing evidence for the implication of spindle misorientation in mammalian diseases, including tumorigenesis and polycystic kidneys. However, the mechanisms regulating spindle orientation in mammals remain largely unknown. The reasons for this include the lack of established approaches in mammalian cells to survey the molecules required for the spindle orientation. We have utilized a simple approach for analyzing spindle orientation in human HeLa cells, in which the mitotic spindles are oriented parallel to the cell-substrate adhesion plane. We used a commercially available siRNA library, which targets 719 genes of human kinases and kinase-related molecules with three individual siRNAs per gene. In the first screening, we scored the spindle orientation of each metaphase cell in a 3-point scale and identified 31 genes as candidates. Then, 31 genes were subjected to the 2nd screening. In the 2nd screening, to quantify the spindle orientation, we measured the angle between the axis of the spindle and that of substratum in metaphase cells. We newly developed an image analyzing software, which automatically detects two poles of mitotic spindles from a series of Z stack images and calculate the spindle angle. We identified 5 genes that are required for the proper spindle orientation in HeLa cells. Inhibition of one of the kinases by the specific chemical inhibitors results in the spindle misorientation in both HeLa cells and in the epithelial cells in the mouse skin, suggesting that this kinase plays an essential role in the spindle orientation not only in the cultured cells but also in the mouse epithelium(submitted).

2) Roles of cholesterol in the progression of mitosis: **M. HAMASAKI and F. TOYOSHIMA**

In this year, we have been investigating the roles of cholesterol in the progression of mitosis. We have found that depletion of cholesterol with methyl-β –cyclodextrin, results in the increases of the proportion of the mitotic cells with multipolar spindles. Moreover, knockdown of squalene synthase, an enzyme essential for the de-novo synthesis of cholesterol, and/ or LDL receptors, also induced multipolar spindles. These results suggest that the cholesterol and/or its metabolites regulate the spindle formation during mitosis.

3) **Regulation of the early endosomes during mitosis: K. IKAWA, S. MATSUMURA, [1]M. FUKUDA and F. TOYOSHIMA** (1Department of Developmental Biology and Neurosciences, Tohoku University)

Early endosomes play central roles in endocytic trafficking. During mitosis, it is known that the fusion and recycling of early endosomes are suppressed. However, the regulatory mechanisms for the early endosomes during mitosis are poorly understood. We have found that one of the mitotic regulators controls early endosome by changing the activity of Rab family proteins, the regulator of early endosomes.

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DEPARTMENT OF CELL BIOLOGY
LABORATORY OF GROWTH REGULATION

The research interest of this laboratory is to understand the molecular mechanism of cell differentiation and organogenesis. Particularly, we are interested in basic helix-loop-helix (bHLH) transcription factors that regulate developmental processes such as neural development and somite formation. We are characterizing the functions of bHLH genes by misexpressing the genes with retrovirus and electroporation (gain-of-function study) and by generating knock-out mice (loss-of-function study). During neural development, the following steps occur sequentially: (1) maintenance of neural stem cells, (2) neurogenesis and (3) gliogenesis. Our results indicate that all three steps are regulated by bHLH genes. bHLH genes such as *Hes1* and *Hes5* regulate maintenance of neural stem cells and promotion of gliogenesis, while bHLH genes such as *Mash1* and *Math3* regulate specification of the neuronal fate. During somite formation, the bHLH genes *Hes1* and *Hes7* display oscillatory expression with periodicity of about two hours and regulate the timing of somite segmentation. By making and evaluating mathematical modeling, we are now studying how the dynamics of gene expression are controlled during somite formation.

Interestingly, new neurons are continuously born from neural stem cells in the adult brain, and it has been shown that this continuous neurogenesis is essential for many brain activities. We found that Notch signaling genes are highly expressed by adult neural stem cells, and that ablation of Rbpj, an essential Noch signaling mediator, leads to depletion of adult neural stem cells and the subsequent loss of adult neurogenesis. Thus, Notch signaling plays an essential role in maintenance of adult neural stem cells and continuation of adult neurogenesis, raising the possibility that Notch signaling genes are therapeutic targets for many brain disorders.

1) Essential roles of Notch signaling in maintenance of neural stem cells in the developing and adult brains: I. IMAYOSHI, M. SAKAMOTO, M. YAMAGUCHI, K. MORI and R. KAGEYAMA

Activation of Notch signaling induces the expression of transcriptional repressor genes such as *Hes1*, leading to repression of proneural gene expression and maintenance of neural stem/progenitor cells. However, a requirement for Notch signaling in the telencephalon was not clear, because in *Hes1;Hes3;Hes5* triple-mutant mice, neural stem/progenitor cells are depleted in most regions of the developing central nervous system, but not in the telencephalon. Here, we investigated a role for Notch signaling in the telencephalon by generating tamoxifen-inducible conditional knock-out mice that lack *Rbpj*, an intracellular signal-mediator of all Notch receptors. When *Rbpj* was deleted in the embryonic brain, almost all telencephalic neural stem/progenitor cells prematurely differentiated into neurons and were depleted. When *Rbpj* was deleted in the

adult brain, all neural stem cells differentiated into transit-amplifying cells and neurons. As a result, neurogenesis increased transiently, but three months later all neural stem cells were depleted and neurogenesis was totally lost. These results indicated an absolute requirement of Notch signaling for the maintenance of neural stem cells and a proper control of neurogenesis in both embryonic and adult brains.

2) Hes1 regulates embryonic stem cell differentiation by suppressing Notch signaling: T. KOBAYASHI and R. KAGEYAMA

Embryonic stem (ES) cells display heterogeneous responses upon induction of differentiation. Recent analysis has shown that *Hes1* expression oscillates with a period of about 3-5 hours in mouse ES cells and that this oscillating expression contributes to the heterogeneous responses: Hes1-high ES cells are prone to the mesodermal fate while Hes1-low ES cells are prone to the neural fate. These outcomes of Hes1-high and Hes1-low ES cells are very similar to those of inactivation and activation of Notch signaling, respectively. These results suggest that Hes1 and Notch signaling lead to opposite outcomes in ES cell differentiation, although they work in the same direction in most other cell types. Here, we found that Hes1 acts as an inhibitor but not as an effector of Notch signaling in ES cell differentiation. Our results indicate that sustained *Hes1* expression delays the differentiation of ES cells and promotes the preference for the mesodermal rather than the neural fate by suppression of Notch signaling.

3) Zinc-finger genes *Fezf1* and *Fezf2* control neuronal differentiation by repressing *Hes5* expression in forebrain: T. SHIMIZU, M. NAKAZAWA, S. KANI, Y.-K. BAE, T. SHIMIZU, R. KAGEYAMA and M. HIBI

Precise control of neuronal differentiation is necessary for generation of a variety of neurons in the forebrain. However, little is known about transcriptional cascades, which initiate forebrain neurogenesis. Here we show that zinc finger genes Fezf1 and Fezf2, which encode transcriptional repressors, are expressed in the early neural stem (progenitor) cells and control neurogenesis in mouse dorsal telencephalon. Fezf1- and Fezf2-deficient forebrains display upregulation of Hes5 and downregulation of neurogenin 2, which is known to be negatively regulated by Hes5. We show that FEZF1 and FEZF2 bind to and directly repress the promoter activity of Hes5. In Fezf1- and Fezf2-deficient telencephalon, the differentiation of neural stem cells into early-born cortical neurons and intermediate progenitors is impaired. Loss of Hes5 suppresses neurogenesis defects in Fezf1- and Fezf2-deficient telencephalon. Our findings reveal that Fezf1 and Fezf2 control differentiation of neural stem cells by repressing Hes5 and, in turn, by derepressing neurogenin 2 in the forebrain.

4) Maximizing functional photoreceptor differentiation from adult human retinal stem cells: T. INOUE, B. COLES, K. DORVAL, R. BREMNER, Y. BESSHO, R. KAGEYAMA, S. HINO, M. MATSUOKA, C. CRAFT, R. MCINNES, F. TEMBLAY, G. PRUSKY, Y. TANO and D. VAN DER KOOY

Retinal stem cells (RSCs) are present in the ciliary margin of the adult human eye and can give rise to all retinal cell types. Here we show that modulation of retinal transcription factor gene expression in human RSCs greatly enriches photoreceptor progeny, and that strong enrichment was obtained with the combined transduction of OTX2 and CRX together with the modulation of CHX10. When these genetically modified human RSC progeny are transplanted into mouse eyes, their retinal integration and differentiation is superior to unmodified RSC progeny. Moreover, electrophysiologic and behavioral tests show that these transplanted cells promote functional recovery in transducin mutant mice. This study suggests that gene modulation in human RSCs may provide a source of photoreceptor cells for the treatment of photoreceptor disease.

5) HES1 and HES5 are dispensable for cartilage and endochondral bone formation: C. KARLSSON, C. BRANTSING, R. KAGEYAMA and A. LINDAHL

Notch signalling, via its downstream mediators HES1 and HES5, regulates development of several different tissues. In vitro studies suggest that these genes are also involved in chondrogenesis and endochondral bone formation. In order to investigate the importance of HES1 and HES5 for these developmental processes, mice lacking chondrogenic expression of HES1 and HES5 were constructed by interbreeding HES5(-/-) mice homozygous for the floxed HES1 allele (HES1(flox/flox)) with COL2A1-Cre transgenic mice, creating conditional HES1;HES5 double mutant mice. The formation of cartilage and endochondral bone was studied in these mice using histological and immunohistochemical stainings, including Alcian Blue van Gieson, Safranin-O, modified Mallory Aniline Blue, tartrate-resistant acid phosphatase and collagen type II stainings. The mice were also studied using several different morphometrical analyses and the differentiation potential of the chondrocytes was evaluated in vitro. Unexpectedly, the conditional HES1;HES5 double mutant mice did not display impaired development of cartilage or endochondral bone. Lack of altered phenotype in the conditional HES1;HES5 double mutant mice can be explained either by the HES1 and HES5 genes not being involved in cartilage and endochondral bone development or by functional redundancy between the genes belonging to the family of HES genes: that is, disruption of one gene could be compensated for by the activity of another. Our results further shed light on the compensatory reserves available during the developing cartilage and bone.

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LABORATORY OF GROWTH REGULATION

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DEPARTMENT OF CELL BIOLOGY
LABORATORY OF SIGNAL TRANSDUCTION

1) Contamination of infectious RD-114 virus in vaccines produced using non-feline cell lines: R. YOSHIKAWA, E. SATO, T. IGARASHI[1] and T. MIYAZAWA ([1]Laboratory of Primate Model, IVR)

All domestic cats have a replication-competent endogenous retrovirus, termed RD-114 virus, in their genome and several feline cell lines produce RD-114 viruses. Recently, we found that a portion of live attenuated feline and canine vaccines produced using feline cell lines was contaminated with infectious RD-114 viruses (Miyazawa *et al*., (2010) J. Virol.). We determined the entire nucleotide sequences of RD-114-related viruses isolated from CRFK cells and a vaccine manufactured using CRFK cells. These RD-114-related viruses were nearly identical to the authentic RD-114 virus (Yoshikawa *et al*., (2010) J. Clin. Microbiol.). In this study, we expanded our survey and examined canine vaccines produced using 'non-feline' cell lines. Consequently, we found two vaccines containing RD-114 viral RNA by reverse transcriptase (RT)-polymerase chain reaction (PCR) and real-time RT-PCR. We also confirmed the presence of infectious RD-114 virus in the vaccines by the LacZ marker rescue assay and PCR to detect proviral DNA in TE671 cells (human rhabdomyosarcoma cells) inoculated with the vaccines. It is impossible to investigate the definitive cause of contamination with RD-114 virus; however, we suspect that a seed canine parvovirus type 2 was contaminated with RD-114 virus, because many canine parvoviruses have been isolated and attenuated using feline cell lines. To exclude RD-114 virus from live attenuated vaccines, we must pay attention to the contamination of seed viruses with RD-114 virus in addition to avoiding feline cell lines producing RD-114 virus when manufacturing vaccines.

2) Susceptibility and production of a feline endogenous retrovirus (RD-114 virus) in various feline cell lines: M. OKADA, R. YOSHIKAWA, T. SHOJIMA, K. BABA and T. MIYAZAWA

RD-114 virus is a replication-competent feline endogenous retrovirus that has been classified as a xenotropic virus. In this study, we examined the expression of the receptors for RD-114 virus in feline cell lines by conducting a pseudotype virus infection assay. Six out of eight feline cell lines were susceptible to the RD-114 pseudotype virus and two cell lines (MCC and FER cells) were resistant. The two resistant cell lines and one cell line (CRFK cells) weakly sensitive to the RD-114 pseudotype virus were found to produce replication-competent RD114-like viruses by the LacZ marker rescue assay and the interference assay. These data strongly suggest that RD-114 virus is polytropic and resistance to RD-114 virus in certain cell lines is due to receptor interference

but not polymorphism of the RD-114 receptors. In addition, we determined the amino acid sequences of the envelope region of RD-114-like viruses produced from MCC, FER and CRFK cells. The sequences were identical with the authentic RD-114 virus. Because many feline cell lines are used to manufacture live attenuated vaccines for companion animals, attention should be paid to contamination of the RD-114 virus in vaccines.

3) **Identification of novel endogenous betaretroviruses which are transcribed in the bovine placenta: K. BABA, Y. NAKAYA, T. SHOJIMA, Y. MUROI[1], K. KIZAKI[2], K. HASHIZUME[2], K. IMAKAWA[1] and T. MIYAZAWA** ([1]Laboratory of Animal Breeding, the University of Tokyo and [2]Laboratory of Veterinary Physiology, Iwate University)

Sequences of retroviral origin occupy approximately 10% of mammalian genomes. Various infectious endogenous retroviruses (ERVs) and functional retroviral elements have been reported for several mammals but not cattle. Here, we identified two proviruses, designated bovine endogenous retrovirus K1 (BERV-K1) and BERV-K2, containing full-length envelope (*env*) genes in the bovine genome. Phylogenetic analysis revealed that they belong to the genus Betaretrovirus. By reverse transcription (RT)-PCR, both BERV-K1 and -K2 *env* mRNAs were detected in the placenta and cultured bovine trophoblast cells. Real-time RT-PCR analysis using RNAs isolated from various bovine tissues revealed that BERV-K1 *env* mRNA was preferentially expressed in the placenta. Moreover, we also found the expression of doubly spliced transcripts, named the REBK1 and REBK2 genes. Both the REBK1 and REBK2 proteins have motifs for a putative nuclear localization signal and a nuclear export signal. REBK1 and REBK2 fused with green fluorescent proteins were localized mainly in the nuclei when they were expressed in bovine and porcine cells. In the *env* and 3' long terminal repeats of BERV-K1 and -K2, we found regulatory elements responsible for the splicing and transport of viral RNAs and/or translation of the *env* genes. Although we have not identified the expressed Env proteins in bovine tissues, these data suggest that both BERV-K1 and BERV-K2 express Env proteins and that these proteins may have physiological functions in vivo.

4) **An endogenous murine leukemia viral genome contaminant in a commercial RT-PCR kit is amplified using standard primers for XMRV: E. SATO, R.A. FURUTA[1] and T. MIYAZAWA** ([1]Japanese Red Cross Osaka Blood Center)

During pilot studies to investigate the presence of viral RNA of xenotropic murine leukemia virus (MLV)-related virus (XMRV) infection in sera from chronic fatigue syndrome (CFS) patients in Japan, a positive band was frequently detected at the expected product size in

negative control samples when detecting a partial gag region of XMRV using a one-step RT-PCR kit. We suspected that the kit itself might have been contaminated with small traces of endogenous MLV genome or XMRV and attempted to evaluate the quality of the kit in two independent laboratories. We purchased four one-step RT-PCR kits from four manufacturers in Japan. To amplify the partial gag gene of XMRV or other MLV-related viruses, primer sets (419F and 1154R, and GAG-I-F and GAG-I-R) which have been widely used in XMRV studies were employed. The nucleotide sequences of the amplicons were determined and compared with deposited sequences of a polytropic endogenous MLV (PmERV), XMRV and endogenous MLV-related viruses derived from CFS patients. We found that the enzyme mixtures of the one-step RT-PCR kit from one manufacturer were contaminated with RNA derived from PmERV. The nucleotide sequence of a partial gag region of the contaminant amplified by RT-PCR was nearly identical (99.4% identity) to a PmERV on chromosome 7 and highly similar (96.9 to 97.6%) to recently identified MLV-like viruses derived from CFS patients. We also determined the nucleotide sequence of a partial *env* region of the contaminant and found that it was almost identical (99.6%) to the PmERV. In the investigation of XMRV infection in patients of CFS and prostate cancer, researchers should prudently evaluate the test kits for the presence of endogenous MLV as well as XMRV genomes prior to PCR and RT-PCR tests.

5) Adaptation of feline immunodeficiency virus subtype B strain TM2 to a feline astrocyte cell line (G355-5 cells): M. ISHIKAWA, K. BABA, M. SHIMOJIMA[1], M. OKADA, T. SHOJIMA, T. MIURA[2] and T. MIYAZAWA ([1]Division of Virology, Department of Microbiology and Immunology, The University of Tokyo and [2]Laboratory of Primate Model, IVR)

Based on receptor usage during infection, feline immunodeficiency virus (FIV) isolates can be divided into two groups; those that require feline CD134 (fCD134) as a primary receptor in addition to CXCR4 to enter the cells, and those that require CXCR4 only. Most primary isolates, including strain TM2, belong to the former group and cannot infect a feline astrocyte cell line (G355-5 cells) due to a lack of fCD134 expression. In a previous study, we found that G355-5 cells transduced with fCD134 (termed G355-5/fOX40 cells) were susceptible to strain TM2 and the inoculated cells became persistently infected. In this study, we examined the phenotype of the virus prepared from the persistently infected cells (termed strain TM2PI). Intriguingly, strain TM2PI replicated well in naïve G355-5 cells and the inoculated G355-5 cells (termed G355-5/TM2PI cells) became persistently infected. The infection of TM2PI in G355-5 cells was inhibited by CXCR4 antagonist AMD3100 and TM2PI infected other fCD134-negative, CXCR4-positive cell lines, FeTJ and 3201 cells. Four amino acid substitutions were found in the Env protein of the strain TM2PI when compared with that of the parental strain TM2. Among the substitutions, the Env amino acid

position at 407 of TM2PI was substituted to lysine which has been known to be responsible for the FIV tropism for Crandell feline kidney cells. The strain TM2PI will be useful for studying the receptor switching mechanism and FIV pathogenesis in cats.

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仲屋友喜、宮沢孝幸：ブタ内在性レトロウイルス受容体の転写制御　第 13 回日本異種移植

研究会、東京、２０１０年３月１４日

宮沢孝幸：内在性レトロウイルスと胎盤形成　第 13 回日本レトロウイルス研究会、愛知、２０１０年８月３１日―９月１日

吉川禄助、佐藤英次、宮沢孝幸：イヌパルボウイルス分離株への RD-114 ウイルスの混入　第 150 回日本獣医学会学術集会、帯広、２０１０年９月１６日―１８日

吉川禄助、下島昌幸、前田　健、宮沢孝幸：RD-114 ウイルスのイヌへの感染性第 150 回日本獣医学会学術集会、帯広、２０１０年９月１６日―１８日

仲屋友喜、星野重樹、庄嶋貴之、安田二朗、宮沢孝幸：ブタ内在性レトロウイルスサブグループ B のエンペロープタンパク質に対する単クローン抗体の作製　第 150 回日本獣医学会学術集会、帯広、２０１０年９月１６日―１８日

宮沢孝幸、吉川禄助、佐藤英次：感染性ネコ内在性レトロウイルス（RD-114 ウイルス）の生ワクチンへの迷入　　第２回動物用ワクチンーバイオ医薬品研究会シンポジウム、帯広、２０１０年９月１８日

星野重樹、大畑拓司、庄嶋貴之、宮沢孝幸：コアラ内在性レトロウイルスの解析　第 58 回日本ウイルス学会学術集会、徳島、２０１０年１１月７日―９日

吉川禄助、下島昌幸、前田　健、宮沢孝幸：ネコ内在性レトロウイルスの異種感染　第 58 回日本ウイルス学会学術集会、徳島、２０１０年１１月７日―９日

仲屋友喜、越　勝男、木崎景一郎、馬場健司、今川和彦、橋爪一善、宮沢孝幸：ウシ内在性レトロウイルスのエンベロープタンパク質の発現と機能解析　第 58 回日本ウイルス学会学術集会、徳島、２０１０年１１月７日―９日

佐藤英次、古田里佳、倉恒弘彦、庄嶋貴之、中富康仁、保井一太、宮沢孝幸：慢性疲労症候群患者血液における xenotropic murine leukemia virus-related virus の核酸調査　第 58 回日本ウイルス学会学術集会、徳島、２０１０年１１月７日―９日

古田里佳、杉山武毅、宮沢孝幸、佐藤英次、倉恒宏彦、中富康仁、保井一太、平山文也：Xenotropic murine leukemia virus-related virus (XMRV) 抗体特異性の解析　第 58 回日本ウイルス学会学術集会、徳島、２０１０年１１月７日―９日

福間藍子、阿部真澄、宮沢孝幸、森川裕子、安田二朗：ネコ Tetherin/BST-2 による RD-114 ウイルスの産生制御　第 58 回日本ウイルス学会学術集会、徳島、２０１０年１１月７日―９日

宮沢孝幸、吉川禄助、佐藤英次：感染性ネコ内在性レトロウイルス（RD-114 ウイルス）の生ワクチンへの迷入　動物用ワクチンーバイオ医薬品研究会シンポジウム、東京、２０１０年１１月７日―９日

仲屋友喜：胎盤形成過程におけるウシ内在性レトロウイルスの役割　第７回　京都大学ウイルス研究所学術交流会、京都、２０１０年１２月１０日

宮沢孝幸：胎盤で機能しうる内在性レトロウイルスの in silico 探索と機能評価　平成２２年度　北海道大学　遺伝子病制御研究所　共同研究集会　「感染・炎症・発癌」、札幌、２０１０年１２月２０日―２１日

CENTER FOR HUMAN RETROVIRUS RESEARCH
LABORATORY OF VIRAL PATHOGENESIS

A long standing goal of our research group is to elucidate the molecular mechanisms of viral infection and pathogenesis. We have been focusing on human viruses, human immunodeficiency virus type 1 (HIV-1), herpes simplex virus type 1 (HSV-1) and Epstein-Barr virus (EBV).

1) Molecular analysis on interaction of HIV-1 protein and host restriction factor: T. KOBAYASHI, K. SATO, Y. SUZUKI, S. YAMAMOTO, P. GEE, T. WATANABE, A. TSUMURA, N. MISAWA, H. EBINA and Y. KOYANAGI

Tetherin, also known as BST-2/CD317/HM1.24, is an antiviral cellular protein that inhibits the release of HIV-1 particles from infected cells. HIV-1 viral protein U (Vpu) is a specific antagonist of human tetherin that might contribute to the high virulence of HIV-1. We recently found that three amino acid (AA) residues (I34, L37, and L41) in the transmembrane (TM) domain of human tetherin are critical for the interaction with Vpu by using a live cell based assay. We also found that conservation of an additional AA at position 45 and two residues downstream of position 22, absent in monkey tetherins, are required for the antagonism by Vpu. Moreover, computer-assisted structural modeling and mutagenesis studies suggest that an alignment of these four AA residues (I34, L37, L41, and T45) on the same helical face in the TM domain is crucial for the Vpu-mediated antagonism of human tetherin. These results contribute to the molecular understanding of human tetherin specific antagonism by HIV-1 Vpu.

We are also attempting to identify HIV-associated host factors using a variety of genetics- and protein chemistry-based methods. Candidates of the HIV-associated host factor under investigation are cytokines, signal molecules, and membrane proteins.

2) Viral pathogenesis: K. SATO, N. MISAWA, T. KOBAYASHI and Y. KOYANAGI

Studies on viral infection in a small animal model that can support both *de novo* human hematopoiesis and systemic viral infection can greatly contribute to the understanding on the pathogenesis. We generated NOG-hCD34 mice by transplanting newborn NOD/SCID/IL2Rγ^{null} mice with hCD34^{+} cells via hepatic injection. EBV-associated hemophagocytic lymphohistiocytosis (EBV-HLH) is a rare yet devastating disorder caused by EBV infection in humans. However, the mechanism of this disease has yet to be elucidated due to a lack of appropriate animal models. We reproduced pathological conditions resembling EBV-HLH in humans using NOG-hCD34 mice. By 10 weeks postinfection, two thirds of the infected mice died after exhibiting high and persistent

viremia, leukocytosis, IFN-γ cytokinenemia, normocytic anemia, and thrombocytopenia. EBV-infected mice also showed systemic organ infiltration by activated $CD8^+$ T cells and prominent hemophagocytosis in bone marrow, spleen, and liver. Notably, the level of EBV load in plasma correlated directly with both the activation frequency of $CD8^+$ T cells and the level of IFN-γ in plasma. Moreover, high levels of EBER1 were detected in plasma of infected mice, reflecting what has been observed in patients. These findings suggest that our EBV infection model mirrors virological, hematological, and immunopathological aspects of EBV-HLH. Furthermore, in contrast to $CD8^+$ T cells, we found a significant decrease of NK cells, MDCs, and PDCs in spleen of infected mice, suggesting that the collapse of balanced immunity associates with the progression of EBV-HLH pathogenesis.

3) HIV integration and it's latency: H. EBINA, Y. KANEMURA and Y. KOYANAGI

Retroelements, such as non LTR-retrotransposon, LTR-retrotransposon and retrovirus, have ability to insert reverse transcribed cDNA into the host chromosome in the replication cycle. Among the retroelements, retrovirus family possesses an efficient DNA-fragment incorporation machinery by acquirement of integrase which catalyzes viral cDNA into chorosome DNA. Because the cDNA integration is essential for the viral replication, the integrase-targeted anti-viral drugs have been created and the clinical investigation of the treated AIDS patients showed significant outcome to reduce viral load after initiation of the drug administration. However, it is well known the non LTR-retrotransposon family of retroelements can insert its genome into the host chromosome through integrase independent machinery such as DNA repair system of non-homologous end joining and/or homologous recombination pathway. It appears that exogenous DNAs have a potential to integrate into the host chromosome. Therefore, we hypothesized that HIV cDNA, which is imported into the nucleus under integrase-depleted condition, can be inserted into host chromosome. To verify this, we prepared the VSV pseudotype lentiviral vector lacking integrase activity with D64V mutation and analyzed the efficiency. As the results, very low efficiency of the cDNA integration, only 0.2% of the event compared to that of wild type integrase vector, could be detected in D64V-infected cells. However, the cDNA insertion of the D64V mutant was clearly increased after treatment with dsDNA break agent before viral infection. Similarly, cDNA integration event and HIV-1 production of WT HIV-1 in the presence of integrase inhibitor could be restored by pre-treatment of target cells with the dsDNA break agent. These results suggest that retroviral cDNA is inserted into the host chromosome through host DNA repair pathway aside from integrase dependent pathway. Furthermore, we found that the HIV-1 provirus generated through integrase independent pathway has a potential to produce progeny viruses.

4) Mechanism of Herpes virus neuropathogenesis: P. GEE, T. WATANABE and Y. KOYANAGI

HSV-1 is a causative agent for fatal encephalitis in human. We generated a HSV-1 encephalitis-survivor model. When a GFP-expressing HSV-1 was inoculated into brain of infant rat, we found that some HSV-1-injected rats (27%; designated as severe) died within 48 hour after showing symptoms of quadriplesia or seizure or some injected rats (22%; designated as mild) died around 72 hour after showing weight loss or paresis. In the brain tissues, we found vast hemorrhagic necrotic damages and largely disseminated GFP^+ region. We detected many GFP^+ cells, which were confirmed as $HSV\text{-}1^+$ cells by staining with anti-HSV antibody along with extensive cell infiltration of $CD3^+$ T cells and $CD68^+$ macrophages, indicating massive dissemination of HSV-1 in brain. However, the other HSV-1-inoculated rats survived after showing the transient mild symptoms such as weight loss or paresis but not quadriplesia or seizure. In the brain tissue taken from recover-rats 36 hour after disappearing paralysis, we found focal neuronal tissue damages along with a small number of cell infiltration of $CD3^+$ T cells and $CD68^+$ macrophages in parenchyma and a fewer GFP^+ cells in the brain of the recover-rats compared with the number of GFP^+ cells in the brain of severe rats. These data indicated that limited but obvious HSV-1 infection occurred in brain of the recover-rats. To find novel host factors that function against HSV-1 infection in brain tissue, we used microarray analysis of messenger RNA comparing that of the recover-rat and the mock-infected brains. Some neuron-specific factors have been found.

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CENTER FOR HUMAN RETROVIRUS RESEARCH
LABORATORY OF VIRUS CONTROL

1) Analyses of *HTLV-1 bZIP factor* (*HBZ*) gene in the pathogenesis of HTLV-1: J. YASUNAGA, Y. SATOU, P. MIYAZATO, T. ZHAO, J. FAN, K. HAGIYA, J. TANABE, K. SUGATA, N. TAGUCHI, A. NAKANISHI, G. MA, Y. MITOBE, A. KAWATSUKI and M. MATSUOKA

Human T-cell leukemia virus type 1 (HTLV-1) is the first retrovirus that induces diseases in human. HTLV-1 causes a neoplastic disease, adult T-cell leukemia (ATL), and the inflammatory diseases, such as HTLV-1 associated myelopathy/tropical spastic paraparesis and uveitis. HTLV-1 belongs to complex retrovirus, which encodes regulatory genes (*tax* and *rex*) and several accessory genes, such as *p30, p12, p13* and *HTLV-1 bZIP factor* (*HBZ*). Among them, *tax* is thought to play a central role in transformation of infected cells. However, since it is a major target of cytotoxic T-lymphocytes, its expression is often silenced in ATL cells to escape the host immune system. The *HBZ* gene, which is encoded by the minus strand of HTLV-1, contains a basic leucine zipper domain. Our previous studies showed that 3'LTR is intact and unmethylated in all ATL cells. From our studies, transcription of the *HBZ* gene was detected in all of the ATL cell lines and primary ATL cases, while *tax* gene transcription was frequently undetectable. In HBZ-transgenic mice, the number of CD4+ T cells was increased, indicating that HBZ promotes proliferation of CD4+ T cells *in vivo*. Dermatitis is frequently observed in HBZ transgenic mice, and in the skin, infiltration of CD4+ T cells is also found. In addition, CD4+ T cells infiltrate into alveolar septum. Among CD4+ T cells increased in HBZ transgenic mice, we found that the population of regulatory T cells (Tregs) was especially increased, and their suppressive function was impaired compared with wild type. Interestingly, HBZ transgenic mice develop T-cell lymphomas more frequently than non-transgenic littermate, and those lymphoma cells expressed a master gene of Tregs, *foxp3*, suggesting the association between HBZ-induced Treg proliferation and its oncogenesis. Those phenotypes of HBZ transgenic mice, such as infiltration of CD4+ T cells to various tissues and onset of T cell lymphomas, are very similar to that of HTLV-1 carriers. These finding suggest that *HBZ* has a crucial role for both leukemogenesis and HTLV-1-associated inflammatory diseases. Furthermore, we are now investigating cellular and molecular mechanism in the HBZ-induced pathogenesis.

2) Identification of cellular proteins interacting with HBZ and characterization of virological and pathological significance of the interaction: J. YASUNAGA, P. MIYAZATO, T. ZHAO, J. FAN, K. HAGIYA, G. MA, A. KAWATSUKI, Y. SATOU and M. MATSUOKA

We are trying to identify cellular factors interacting with HBZ by using yeast two hybrid or functional analyses of various signaling pathways. In this study, we found that HBZ specifically suppressed NF-κB-driven transcription mediated by p65 and tax but not the alternative NF-kB signaling pathway. Using coimmunoprecipitation, we demonstrated the direct interaction between HBZ and p65, and this physical association abrogated the DNA binding capacity of p65. In other aspect, HBZ induced p65 degradation through ubiquitination-dependent pathway. In addition, HBZ repressed transcription of selected classic NF-κB target genes. This study suggests that this selective binding to p65 modulates Tax mediated NF-κB activation. We got other cellular candidates interacting with HBZ using yeast two hybrid screening. We are investigating their significances in pathogenesis of HTLV-1-induced diseases.

3) Nonsense mutations in HTLV-1-encoded genes and their significances in leukemogenesis of HTLV-1-infected cells: J. FAN, G. MA and M. MATSUOKA

Genetic changes in the *tax* gene in ATL cells were reported in about 10% of ATL cases. To determine genetic changes that may occur throughout the provirus, we determined the entire sequence of the HTLV-1 provirus in 60 ATL cases. Abortive genetic changes, including deletions, insertions, and nonsense mutations, were frequent in all viral genes except the *HBZ* gene, suggesting that HBZ is critical in the process of ATL genesis. G-to-A base substitutions were the most frequent mutations in the provirus of ATL cells. The sequence context of G-to-A mutations was in accordance with the preferred target sequence of human APOBEC3G (hA3G). The target sequences of hA3G were less frequent in the plus strand of the *HBZ* coding region than in other coding regions of the HTLV-1 provirus. Thus, HBZ can escape mutations by hA3G since it is encoded in the minus strand.

4) Characterization of DNA repair proteins involved in retroviral integration: Y. SAKURAI and M. MATSUOKA

Retrovirus synthesizes viral dsDNA by reverse transcription and integrates the DNA into the host genome by integration. There are virus-specific preferences in retroviral integration sites. Murine leukemia virus (MLV) prefers genomic regions near transcriptional start sites, CpG islands and DNase hypersensitive sites for its integration, while the molecular mechanism for this preference remains unknown. In this study, we analyzed a huge number of the integration sites by massively parallel sequencing, and found that mutant cells lacking a DNA repair protein NBS1 showed decreased MLV integration frequency near transcriptional start sites, CpG islands and DNase hyper sensitive sites compared to NBS1-complemented cells. NBS1-deficient cells also showed decreased integration within H3K4me3 and H3K9me1 regions, which are epigenetic marks

associated with active promoters. In contrast, the NBS1-deficient cells showed increased integration within H3K79me3 regions, which are detected near silent promoters. Moreover, we demonstrated physical interaction of NBS1 and viral DNA before integration in MLV-infected cells by using ChIP assay. This study indicates that a DNA repair protein NBS1 is a host factor controlling MLV integration targeting.

5) Resistance mechanism to the next-generation HIV-1 fusion inhibitors: K. SHIMURA and M. MATSUOKA

Enfuvirtide (T-20), an HIV-1 gp41-derived peptide, efficiently inhibits HIV infection. However, effective therapy has been hindered by the emergence of resistant variants. We have developed two potential second-generation fusion inhibitors (FIs), SC34 and SC34EK, rationally designed to stably form six-helix bundles. We reported that SC34 and SC34EK selected several mutations in gp41, which were required to confer resistance to both FIs. In addition to the gp41 mutations, SC34 and SC34EK selected several mutations in gp120. Although these mutations did not confer any resistance to FIs by themselves, they enhanced the resistance caused by SC34- and SC34EK-selected gp41 mutations to T-20. SC34- and SC34EK-selected mutations in gp120 enhanced the replication kinetics attenuated by the gp41 mutations. The multiple mutations in the gp41 are necessary for the resistance to SC34 and SC34EK, while those in gp120 act as secondary mutations that restore replication kinetics diminished by the gp41 mutations.

6) Development of novel small-molecule inhibitors for HIV: K. SHIMURA, H. TOGAMI, T. ISOBE, T. NAITO and M. MATSUOKA

Highly active anti-retroviral therapy (HAART) potently suppresses viral replication, and improves prognosis of HIV-1 infected individuals. However, long-term usage of anti-retrovirus drugs allows emergence of resistant viruses. In order to develop novel small-molecule HIV inhibitors, we screened more than 30,000 compounds and identified several that showed anti-HIV activity by inhibiting the early-phase of HIV replication cycle. Among them, some compounds target steps other than attachment/fusion, reverse transcription, or integration, suggesting that a novel mode of action is involved in their activity. We will further enhance their anti-HIV activity and identify the mechanism of action.

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EXPERIMENTAL RESEARCH CENTER FOR INFECTIOUS DISEASES
LABORATORY OF MOUSE MODEL

Our research objective is to understand the molecular mechanisms that control chromatin function and genome diversity & stability in mammals. To address this question, we are currently analyzing functional molecules which are expressed in the nucleus.

1) Roles of the histone lysine demethylases Jmjd1a and Jmjd1b in murine embryonic development: M. TACHIBANA and Y. SHINKAI

Histone H3 lysine 9 (H3K9) methylation is a repressive epigenetic mark for heterochromatin formation and transcriptional silencing. Our research purpose is to understand the dynamics of H3K9 methylation in mammalian development and to indentify the molecule(s) that regulate H3K9 methylation. We previously showed that coordinated expression of the H3K9 methylatransferase G9a and the H3K9 demethylase Jmjd1a dynamically regulate H3K9 methylation levels during male meiosis in mice (Tachibana et al., 2007). To elucidate further molecular function of Jmjd1a during murine development, we have established *Jmjd1a* knockout (KO) mice. *Jmjd1a*-KO mice are born at a Mendelian ratio and become adult, suggesting that Jmjd1a is not essential for embryonic development. However, methylation levels of H3K9me2 are not significantly chanced in *Jmjd1a*-KO embryos. Jmjd1a has two homologues, Jmjd1b and Jmjd1c in mammals, and Jmjd1b can also catalyze H3K9 demethylation. mRNA and protein for *Jmjd1a* and *Jmjd1b* genes are expressed similarly in post-implantation embryos, suggesting the redundant function(s) of these two enzymes. To examine the functional redundancy between Jmjd1a and Jmjd1b, first we established *Jmjd1b*-KO mice by a conventional KO strategy. *Jmjd1b*-KO mice were born at sub-Mendelian ratio and no morphological abnormalities were detected between wild-type and *Jmjd1b*-KO embryos at E12.5, suggesting that Jmjd1b is also not essential for murine embryogenesis until med-gestation. Again, methylation levels of H3K9me2 were indistinguishable between wild-type and *Jmjd1b*-KO embryos. To investigate a redundant role of Jmjd1a and Jmjd1b on mouse embryogenesis, mice carrying both *Jmjd1b+/-* and *Jmjd1b+/-* alleles were intercrossed. We could not obtain offspring carrying both *Jmjd1a-/-* and *Jmjd1b-/-* alleles, suggesting that *Jmjd1a/b* double KO (DKO) mice are embryonic lethal. So far, no *Jmjd1a/b*-DKO embryos were detectable even at E7.5 stage. To summarize, it was revealed that Jmjd1a and Jmjd1b are redundantly essential for mouse embryogenesis. Currently we are identifying the lethal point of *Jmjd1a/b*-DKO embryos and examining these phenotypes including the status of H3K9 methylation.

2) Analysis of epigenetic regulation of mammalian sex differentiation: M. TACHIBANA

Sex differentiation is the process of development of the differences between males and females from an undifferentiated zygote. This event is essential for sexually reproducing organisms to pass a combination of genetic material to offspring, resulting in increased genetic diversity. In mammal, the presence of *Sry* in the bipotential fetal gonads switches the developmental program into testes (Koopman et al., 1991). Once sex has been established, that is maintained throughout the life. It is still unknown how epigenetic machinery contributes to this sex determination process. In addition, the responsible enzymes are unclear which contribute the epigenetic maintenance of sex specific gene-expression profiles. To gain insight of these phenomena, we are planning to analyze the sex-specific epigenome structure in mammalian gonadal somatic cells. To purify mice gonadal somatic cells, we designated transgenic (TG) mice that express human low-affinity nerve growth factor receptor (LNGFR) without intracellular domains. We chose Ad4BP/SF1 promoter to drive LNGFR, since Ad4BP/SF1 is specifically expressed embryonic gonadal somatic cells from E10.5 onwards in both sexes. Bacmids containing all exons for Ad4BP/SF1 were modified in order to generate TG mice. Briefly, ATG sequences corresponding to start codon of Ad4BP/SF1 were replaced with LNGFR open reading frame. The modified bacmids were injected into pronucleus in C57BL/6 zygote. Finally, it was found that three lines of offspring carried the integrated bacmids . Among them, mRNA and protein of LNGFR were strongly expressed in embryonic gonad in two-lines. Immunohistochemical analyses reveals the LNGFR protein were expressed in gonadal somatic lineage but not in germ cells, suggesting successful generation of Ad4BP/SF1-LNGFR TG lines. Next, we performed the purification of gonadal somatic cells using anti-LNGFR antibodies and magnetic separation system. More than 95% cells purified were positive for Ad4BP/SF1 protein. Currently we are planning to optimize this purification process.

3) Analysis of the role of histone modification in DNA damage repair: T. TSUBOTA and Y. SHINKAI

Histone modification is required for not only transcriptional regulation but also DNA repair. After DNA damage, checkpoint protein ATM is rapidly activated and cell cycle checkpoint and DNA repair are stimulated. It has been reported that when DNA damage is occurred in heterochromatin, ATM activation requires H3K9 methylation catalyzed by SUV39H1/H2 enzymes (*Nature Cell Biology*, p1376, 2009). However, DNA damage is often occurred in euchromatin, and its chromatin regulation is almost unknown. Since the characteristics are quite different between heterochromatin and euchromatin, the regulation with DNA damage could be also different. Therefore, we tested this hypothesis by using *G9a* knockout (KO) mouse ES cells that reduce H3K9 methylation mainly in euchromatin. Although *Suv39h1/h2*-DKO cells showed the delay of ATM activation with DNA damage, *G9a*-KO cells showed the stimulation. Furthermore, H3K56 acetylation which is important for DNA repair in yeast is accumulated in *G9a*-KO cells but not in

Suv39h1/h2-DKO cells, suggesting some crosstalk may exist between H3K9 methylation in euchromatin and H3K56 acetylation. Since the accumulation of H3K56 acetylation is also recently reported in several cancers, we will focus on to elucidate the molecular mechanism of the abnormal histone modification pattern and ATM activation seen in *G9a*-KO cells, in comparison with cancer cells. Knockout of other H3K9 methyltransferase, ESET does not alter the ATM activation pattern. However, *ESET*-KO cells show the higher protein expression level of p53 and are more resistant against a DNA damage agent. We would like to reveal this mechanism, too.

4) Profiling of histone lysine methylation during mouse germ cell development: K. DEGUCHI and Y. SHINKAI

Somatic and germ cells develop during embryogenesis. Somatic cell is required for maintenance of homeostasis and germ cell is essential for maintenance of species. Our laboratory studies the role of histone post-translational modifications in biological processes. Many reports suggest that histone methylation is needed for germ cell development and functions.

We analyzed histone lysine methylation status in germ-lineage cells during embryogenesis after sex determination. We found that the methylation status of multiple histone lysine residues are different between male and female. Especially, global level of histone H3 lysine 9 dimethylation (H3K9me2) is low in male germ-lineage cells. In mouse, histone lysine methyltransferases G9a and GLP form a heteromeric complex and cooperatively regulate H3K9me2 and me1. GLP but not G9a is extremely low in male germ-lineage cells at the developmental stages we examined. It was reported that *Glp* mRNA is not expressed in primordial germ cells before sex determination. However, *Glp* mRNA is detected in male germ-lineage cells after sex determination as that in somatic cells. Interestingly, GLP is also not detectable in undifferentiated spermatogonia which is reported to have low H3K9me2. We suggest that H3K9me2 is not deposited in embryonic male germ-lineage cells and undifferentiated spermatogonia since GLP protein expression is suppressed by post-transcriptional regulation. We are currently investigating this suppression mechanism.

LIST OF PUBLICATIONS
EXPERIMENTAL RESEARCH CENTER FOR INFECTIOUS DISEASES
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EXPERIMENTAL RESEARCH CENTER FOR INFECTIOUS DISEASES
LABORATORY OF PRIMATE MODEL

It has been 27 years since human immunodeficiency virus (HIV-1), the causative agent of acquired immune deficiency syndrome (AIDS) was first identified. Since then, our knowledge on HIV-1 and the pathophysiology of AIDS has grown enormously. Unfortunately, however, we have not yet developed an effective prophylactic measure or a thorough therapeutic intervention, and AIDS remains top priority among global public health agenda.

To develop effective preventive or therapeutic measures against AIDS, we need an experimental model system that recapitulates HIV-1 infection in humans. From the beginning of AIDS epidemic, HIV-1 has been known for its narrow host range. To overcome the narrow host range of HIV-1 and develop a dependable animal model for AIDS, our laboratory, first in the world, generated a chimeric simian-human immunodeficiency virus (SHIV), that carries HIV-1 derived *tat*, *rev*, *vpu* and *env* genes in the backbone of simian immunodeficiency virus, a closely related simian virus to HIV-1. Since then, SHIV/macaque model has been further developed and there are currently several SHIV strains available in the field and some of them cause acute disease followed by AIDS-like clinical manifestations.

We have been pursuing the following subjects,

1. Development and improvement of SHIV/macaque models,
2. SHIV-induced pathogenesis,
3. Development of novel vaccines and evaluation using SHIV/macaque system,
4. Identification of virus reservoir in HIV-1 infected individuals under highly active anti-retroviral therapy (HAART) using SIV infected monkeys as a model.

In addition to the abovementioned projects, we have been making efforts to establish non-human primate disease model for flavivirus infection, especially, dengue hemorrhagic fever.

1) Generation of the pathogenic R5-tropic simian/human immunodeficiency virus SHIVAD8 by serial passaging in rhesus macaques: Y. NISHIMURA, M. SHINGAI, R. WILLEY, R. SADJADPOUR, W. R. LEE, C. R. BROWN, J. M. BRENCHLEY, A. BUCKLER-WHITE, R. PETROS, M. ECKHAUS, V. HOFFMAN, T. IGARASHi and M. A. MARTIN

A new pathogenic R5-tropic simian/human immunodeficiency virus (SHIV) was generated following serial passaging in rhesus macaques. All 13 animals inoculated with SHIVAD8 passaged lineages experienced marked depletions of $CD4^+$ T cells. Ten of these infected monkeys became normal progressors (NPs) and had gradual losses of both memory and naïve $CD4^+$ T lymphocytes, generated antiviral $CD4^+$ and $CD8^+$ T cell responses, and sustained chronic immune activation

while maintaining variable levels of plasma viremia (10^2 to 10^5 RNA copies/ml for up to 3 years postinfection [p.i.]). To date, five NPs developed AIDS associated with opportunistic infections caused by *Pneumocystis carinii*, *Mycobacterium avium*, and *Campylobacter coli* that required euthanasia between weeks 100 and 199 p.i. Three other NPs have experienced marked depletions of circulating $CD4^+$ T lymphocytes (92 to 154 cells/μl) following 1 to 2 years of infection. When tested for coreceptor usage, the viruses isolated from four NPs at the time of their euthanasia remained R5 tropic. Three of the 13 SHIVAD8-inoculated macaques experienced a rapid-progressor syndrome characterized by sustained plasma viremia of $>1 \times 10^7$ RNA copies/ml and rapid irreversible loss of memory $CD4^+$ T cells that required euthanasia between weeks 19 and 23 postinfection. The sustained viremia, associated depletion of $CD4^+$ T lymphocytes, and induction of AIDS make the SHIVAD8 lineage of viruses a potentially valuable reagent for vaccine studies.

2) Small intestine $CD4^+$ cell reduction and enteropathy in simian/human immunodeficiency virus KS661-infected rhesus macaques in the presence of low viral load: K. INABA, Y. FUKAZAWA, K. MATSUDA, A. HIMENO, M. MATSUYAMA, K. IBUKI, Y. MIURA, Y. KOYANAGI, A. NAKAJIMA, R. S. BLUMBERG, H. TAKAHASHI, M. HAYAMI, T. IGARASHI and T. MIURA

Human immunodeficiency virus type 1, simian immunodeficiency virus and simian/human immunodeficiency virus (SHIV) infection generally lead to death of the host accompanied by high viraemia and profound CD4+ T-cell depletion. SHIV clone KS661-infected rhesus macaques with a high viral load set point (HVL) ultimately experience diarrhoea and wasting at 6–12 months after infection. In contrast, infected macaques with a low viral load set point (LVL) usually live asymptomatically throughout the observation period, and are therefore referred to as asymptomatic LVL (Asym LVL) macaques. Interestingly, some LVL macaques exhibit diarrhoea and wasting similar to the symptoms of HVL macaques and are termed symptomatic LVL (Sym LVL) macaques. This study tested the hypothesis that Sym LVL macaques have the same degree of intestinal abnormalities as HVL macaques. The proviral DNA loads in lymphoid tissue and the intestines of Sym LVL and Asym LVL macaques were comparable and all infected monkeys showed villous atrophy. Notably, the CD4+ cell frequencies of lymphoid tissues and intestines in Sym LVL macaques were remarkably lower than those in Asym LVL and uninfected macaques. Furthermore, Sym LVL and HVL macaques exhibited an increased number of activated macrophages. In conclusion, intestinal disorders including CD4+ cell reduction and abnormal immune activation can be observed in SHIV-KS661-infected macaques independent of virus replication levels.

3) ***In vivo*** **analysis of a new R5 tropic SHIV generated from the highly pathogenic SHIV-KS661, a derivative of SHIV-89.6: K. MATSUDA, K. INABA, Y. FUKAZAWA, M. MATSUYAMA, K. IBUKI, M. HORIIKE, N. SAITO, M. HAYAMI, T. IGARASHI and T. MIURA**

Although X4 tropic SHIVs have been studied extensively, they show distinct infection phenotypes from those of R5 tropic viruses, which play an important role in HIV-1 transmission and pathogenesis. To augment the variety of R5 tropic SHIVs, we generated a new R5 tropic SHIV from the highly pathogenic X4 tropic SHIVKS661, a derivative of SHIV-89.6. Based on consensus amino acid alignment analyses of subtype B R5 tropic HIV-1, five amino acid substitutions in the third variable region successfully changed the secondary receptor preference from X4 to R5. Improvements in viral replication were observed in infected rhesus macaques after two passages, and reisolated virus was designated SHIV-MK38. SHIV-MK38 maintained R5 tropism through *in vivo* passages and showed robust replication in infected monkeys. Our study clearly demonstrates that a minimal number of amino acid substitutions in the V3 region can alter secondary receptor preference and increase the variety of R5 tropic SHIVs.

4) **Evaluation of the immune response and protective effects of rhesus macaques vaccinated with biodegradable nanoparticles carrying gp120 of human immunodeficiency virus: A. HIMENO, T. AKAGI, T. UTO, X. WANG, M. BABA, K. IBUKI, M. MATSUYAMA, M. HORIIKE, T. IGARASHI, T. MIURA and M. AKASHI**

We previously reported that biodegradable amphiphilic poly(γ-glutamic acid) nanoparticles (NPs) carrying the recombinant gp120 env protein of the human immunodeficiency virus type 1 (HIV-1) were efficiently taken up by dendritic cells, and induced strong $CD8^+$ T cell responses against the gp120 in mice. To evaluate gp120-carrying NPs (gp120-NPs) as a vaccine candidate for HIV-1 infection, we vaccinated rhesus macaques with these gp120-NPs and examined theimmuneresponse and protective efficacy against a challenge inoculation of simian and human immunodeficiency chimeric virus (SHIV). We found that gp120-NP vaccination induced stronger responses for both gp120-specific cellular and humoral immunity than gp120-alone vaccination. After the challenge inoculation with SHIV, however, the peak value of viral RNA in the peripheral blood was higher in the vaccinated groups, especially the gp120-NP vaccinated group, than naive control group. Higher value of viral load was also maintained in gp120-NP vaccinated group. Furthermore, $CD4^+$ T cells from the peripheral blood decreased more in the vaccinated groups than the control group. Thus, induced immune responses against gp120 enclosed in NPs were not effective for protection but, conversely enhanced the infection, although the gp120-NPs showed a

stronger induction of immune responses against the vaccinated antigen in rhesus macaques. These results support the importance of determining immune correlate of protective immunity for vaccine development against HIV-1 infection.

5) An improved reverse genetics system for mammalian orthoreoviruses: T. KOBAYASHI, L. S. OOMS, M. IKIZLER, J. D. CHAPPELL and T. S. DERMODY

Mammalian orthoreoviruses (reoviruses) are highly useful models for studies of double-stranded RNA virus replication and pathogenesis. We previously developed a strategy to recover prototype reovirus strain T3D from cloned cDNAs transfected into murine L929 fibroblast cells. Here, we report the development of a second-generation reovirus reverse genetics system featuring several major improvements: (1) the capacity to rescue prototype reovirus strain T1L, (2) reduction of required plasmids from 10 to 4, and (3) isolation of recombinant viruses following transfection of baby hamster kidney cells engineered to express bacteriophage T7 RNA polymerase. The efficiency of virus rescue using the 4-plasmid strategy was substantially increased in comparison to the original 10-plasmid system. We observed full compatibility of T1L and T3D rescue vectors when intermixed to produce a panel of T1LxT3D monoreassortant viruses. Improvements to the reovirus reverse genetics system enhance its applicability for studies of reovirus biology and clinical use.

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中村仁美、大附寛幸、松田健太、小林剛、五十嵐樹彦、三浦智行：相同組換えによって作製した新規サル指向性ヒト免疫不全ウイルスの遺伝子解析、第 24 回日本エイズ学会学術集会、東京、2010 年 11 月 24-26 日

藤田泰久、大附寛幸、小林剛、三浦智行、五十嵐樹彦：新規組換え技術による CCR5 指向性 clade C HIV-1 株の env 領域を持った SHIV の作製、第 24 回日本エイズ学会学術集会、東京、2010 年 11 月 24-26 日

CENTER FOR EMMERGING VIRUS RESEARCH

1) HIV and EBV Pathogenesis: K. SATO, N. MISAWA and Y. KOYANAGI

Human APOBEC3G was identified as an HIV-1 restriction factor, which edits nascent HIV-1 DNA by inducing G-to-A hypermutations and debilitates the infectivity of *vif*-deficient HIV-1. On the other hand, HIV-1 Vif protein has the robust potential to degrade APOBEC3G protein. Although following investigations have revealed that lines of APOBEC3 family proteins have the capacity to mutate HIV-1 DNA, it remains unclear whether these endogenous APOBEC3s including APOBEC3G contribute to mutations of *vif*-proficient HIV-1 provirus *in vivo*. We use NOG-hCD34 mouse model and demonstrate the predominant accumulation of G-to-A mutations in *vif*-proficient HIV-1 provirus displaying characteristics of APOBEC3-mediated mutagenesis. Notably, the APOBEC3-associated G-to-A mutation of HIV-1 DNA that leads to the termination of translation was significantly observed. We further provide a novel insight suggesting that HIV-1 G-to-A hypermutation is independently induced by individual APOBEC3 protein. In contrast to the observation in proviral DNA, viral RNA possessed less G-to-A mutations. Taken together, these results provide the evidence indicating that endogenous APOBEC3s are associated with G-to-A mutation of HIV-1 provirus *in vivo*, which can result in the abrogation of HIV-1 replication.

2) Role of the envelope stress response system in the biosynthesis and quality control of envelope proteins. S. NARITA and Y. AKIYAMA[1] ([1]Department of Viral Oncology, IVR)

The aim of research in this group is to clarify the survival strategy of gram-negative bacteria. Various bacterial species in this phylum have been identified as causative microorganisms of many infectious diseases. It is of great importance, therefore, to understand their survival strategy to cope with emerging infectious diseases. Cell structure of gram-negative bacteria is characterized by the presence of the outer membrane surrounding the cytoplasmic membrane and the periplasmic space. These envelope structure functions as a permeability barrier against toxic compounds and serves to maintain homeostasis of the cytoplasm. Because the outer membrane is essential for the growth of gram-negative bacteria, knowledge of the biosynthesis and quality control systems of the outer membrane would contribute to develop new drugs against gram-negative pathogenic bacteria. We study these systems using *Escherichia coli*, the model organism that has ever been most extensively studied.

The σ^E stress response system senses misfolded outer membrane proteins (OMPs) in the

periplasmic space and regulates expression of a set of genes that cope with envelope stresses. Upon activation of σ^E, expression of genes for periplasmic chaperones/proteases and components for OMP and lipopolysaccharide assemblies are up-regulated while those for OMPs are down-regulated, both contributing to reduce the threat to periplasmic accumulation of misfolded OMPs. Although there are several genes encoding periplasmic proteases that are up-regulated upon the activation of σ^E, their physiological roles in the stress response are not fully understood. We found that an *E. coli* strain deleted for a σ^E-regulated putative periplasmic metalloprotease exhibits increased sensitivity to an anionic detergent, SDS, and several antibiotics including erythromycin and rifampicin, indicating that this protease is required to maintain the integrity of the outer membrane. Furthermore, deleterious effects of loss of this protease were synergistically increased by additional disruption of either *bamB* or *surA*, genes encoding a component of the OMP assembly machinery and a periplasmic chaperon, respectively. These results suggested a novel function of a σ^E-regulated protease involved in quality control of OMPs through its proteolytic activity.

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佐藤佳, 岩見真吾, 三沢尚子, 伊藤守, 小柳義夫. ヒト化マウス末梢血による DNA ラベリング系の確立 -動物実験- , 第 20 回日本数理生物学会大会, 札幌, 2010 年 9 月 13 日―16 日

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佐藤佳, 三沢尚子, Chuanyi Nie, 佐藤賢文, 松岡雅雄, 高橋玲, 伊藤守, 高田賢蔵, 小柳義夫. ヒト化マウスを用いた EBV 関連血球貪食性リンパ組織球症モデルマウスの確立, 第 58 回日本ウイルス学会学術集会, 徳島, 2010 年 11 月 7 日-9 日

小林朋子、芳田剛、佐藤佳、Peter Gee、山元誠司、蝦名博貴、小柳義夫 HIV-1 Vpu 相互作用に必須な tetherin/Bst-2 膜貫通領域アミノ酸の同定, 第 58 回日本ウイルス 学会学術集会、徳島、2010 年 11 月 7 日-9 日

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2) Second Group

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Tao, K., Watanabe, S., Narita, S. and Tokuda, H. A periplasmic LolA derivative with a lethal disulfide bond activates the Cpx stress response system. J. Bacteriol. 192, 5657-5662, 2010.

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成田新一郎：グラム陰性細菌の細胞表層形成に関与する ABC トランスポーターの研究. 日本農芸化学会 2010 年度大会、東京、２０１０年３月２７日

成田新一郎、徳田元：細菌リポタンパク質の外膜局在化を司る ABC トランスポーター LolCDE の機能. 第 5 回トランスポーター研究会年会、東京、２０１０年７月１１日

成田新一郎：グラム陰性細菌の細胞表層形成に関与する ABC トランスポーターの研究. 日本農芸化学会関東支部 2010 年度第１回支部例会、筑波、２０１０年７月１７日

Narita, S. and Tokuda, H. Overexpression of LolCDE suppresses the growth defect of an *Escherichia coli* mutant that lacks apolipoprotein N-acyltransferase. The 3rd International Symposium on Protein Community. September 13-16. Nara, 2010.

REPRODUCTIVE ENGINEERING TEAM

Reproductive engineering team is a support unit for generating transgenic mouse (Tg) and knockout mouse (KO) under the animal committee of our institute. We also perform cryopreservation of mouse fertilized eggs. Current staffs are Kitano and Miyachi. Results of last three years are as follows.

1) Freezing embryos

2008	62 strains	20,525 embryos
2009	75 strains	20,337 embryos
2010	101 strains	18,620 embryos

2) Introduction of mouse strains from outside

	Frozen embryos	Live mice
2008	4 strains	2 strains
2009	7 strains	2 strains
2010	4 strains	6 strains

3) Transgenic mouse production with cloned DNAs

	No of constructs	No of embryos injected	No of transgenic pups obtained
2008	52	20,379	125 (0.6%)
2009	94	33,821	190 (0.6%)
2010	90	32,875	124 (0.3%)

4) Production of chimeric mouse

	No of ES clones	No of embryos injected	No of coatcolor chimera obtained
2008	49	7,252	357 (4.9%)
2009	52	4,587	242 (5.3%)
2010	106	7,106	394 (5.5%)

LIST OF PUBLICATIONS
REPRODUCTIVE ENGINEERING TEAM

Takeo, T., Kondo, T., Haruguchi, Y., Fukumoto, K., Nakagawa, Y., Takeshima,Y., Nakamuta, Y., Tsuchiyama, S., Shimizu, N., Hasegawa, T., Goto, M., Miyachi, H., Anzai, M., Fujikawa, R., Nomura, K., Kaneko. T., Itagaki, Y., Nakagata, N. Short-term Storage and Transport at Cold Temperatures of 2-Cell Mouse Embryos Produced by Cryopreserved Sperm. Journal of the American Association for Laboratory Science.49, 1-5, 2010.

Matsui, T., Leung, D., Miyashita, H., Maksakova, IA., Miyachi, H., Kimura, H., Tachibana, M., Lorincz, MC., Shinkai, Y. Proviral silencing in embryonic stem cells requires the histone methyltransferase ESET. Nature. 464, 927-31, 2010.

宮地　均、北野　さつき、竹尾　透、福本紀代子、近藤朋子 2、春口幸恵、竹下由美、中牟田裕子、土山修治、金子武人、眞貝　洋一、中潟　直己: 凍結融解マウス２細胞期胚の低温保存（４℃）に関する研究. 第 57 回日本実験動物学会総会、京都、２０１０年 5 月 12 日～14 日

宮地　均、北野　さつき、眞貝　洋一： 海外ノックアウトプロジェクトから導入した ES 細胞からのキメラマウス作製の実際. 第 44 回日本実験動物技術者協会、旭川、２０１０年 9 月 3 日・４日

COMPUTER NETWORK OF INSTITUTE FOR VIRUS RESEARCH

Institute for Virus Research LAN system (IVR-LAN) has administrated by the network committee consisted of four staffs (Prof. Toyoshima, Prof. Akiyama, Associate Prof. Mori and Instructor Takemoto). IVR-LAN service has covered for researchers of some medical departments as well as IVR, and the primary purpose of IVR-LAN is to offer accessibility to the Internet in support of their studies.

IVR-LAN has provided a variety of network services, including E-Mail, WEB-mail, WWW, File-sharing, SSH and all Outgoing TCP services except for P2P. Main services are working on Sun Sparc platform with Solaris 10 and DELL with Linux.

All servers had been settled in a neighboring building during repair work which had made our institute earthquake-resistant. We started Juniper SSL VPN service on purpose to maintain our services including the exclusive ones such as a license management during relocation. It ensured the connection into IVR-LAN for users outside our network. Though there were shutdowns of all servers twice a year, we were able to come back to home building without serious network troubles.

This year we have begun MAC address filtering, which means you have to register the MAC address belonging to your personal computer in order to connect into IVR-LAN. Because the number of Wi-Fi devices and wireless users are increasing significantly nowadays, a rogue hotspot could lead to open up security holes. However IVR-LAN has adequately equipped, we must have a responsibility for sending/getting data. A few accidents have occurred in this year. IVR-LAN users need to get certifications of training of e-learning course which is provided by Institute for Information Management and Communication of Kyoto university.

STAFF CHANGES OF THE INSTITUTE

Appointments

During the period of January to December 2010, the following new staffs were appointed; Dr. Yoshihiro Kawaoka as a Visiting Professor of Department of Biological Responses, Dr. Masafumi Takiguchi as a Visiting Professor of Laboratory of Viral Immunology, Dr. Katsuji Sugie as a Visiting Associate Professor of Department of Biological Responses, Dr. Hiroki Kato as an Associate Professor of Department of Genetics and Molecular Biology, Dr. Jun-ichiro Yasunaga as a Lecture of Center for Human Retrovirus Research, Dr. Hirotaka Kuwata as an Assistant Professor of Department of Viral Oncology, Drs. Kei Sato, Shin-ichiro Narita and Ayano Satsuka as an Assistant Professor of Center for Emerging Virus Research, Drs. Toru Kiyono and Yasuhito Tanaka as a Lecture (part time) of Department of Viral Oncology, Drs. Yoshiharu Matsuura and Hisashi Arase as a Lecture (part time) of Department of Genetics and Molecular Biology, Dr. Junji Takeda as a Lecture (part time) of Department of Biological Responses, Dr. Hiroaki Takeuchi as a Department of Cell Biology, Drs. Yusuke Yanagi, Takeshi Noda, Kyoko Shinya and Michinori Kohara as a Lecture (part time) of Center for Human Retrovirus Research, Dr. Koichi Morita as a Lecture (part time) of Experimental Research Center for Infectious Diseases.

Departure

Dr. Mitsutoshi Yoneyama moved to Medical Mycology Research Center, Chiba University, Dr. Eiji Ido moved to Tokyo Medical and Dental University, Dr. Yoichi Suzuki moved to National University of Singapore, Dr. Shinobu Chiba moved to Kyoto Sangyo University, Dr. Aoi Son moved to National Center for Global Health and Medicine, Dr. Junji Yodoi retired from the Institute in March, Drs. Yousuke Takahama, Hideyuki Tanabe, Hironori Niki, Hideki Nishitoh, Tetsuro Matano, Jun Nishihira, Masaaki Miyazawa, Hiroyuki Mano, Takeshi Imamura, Tomohiko Takasaki, Ryuzo Torii, Chieko Kai left the Institute. 2010

THE SCIENTIFIC LECTURES OF THE INSTITUTE FOR VIRUS RESEARCH

The annual scientific lecture of this Institute was held on July 15, 2010 at the Kyoto University Hall.

Program

Opening Remarks: Masao Matsuoka

1. Mechanism of pathogenesis by HTLV-1 bZIP factor, Masao Matsuoka, this Institute

2. HCV, replication in cell culture and mechanism of chronic infection in vivo, Takaji Wakita, National Institute of Infectious Diseases

3. Regulation of neural stem cells : Brain formation and maintenance, Ryoichiro Kageyama, this Institute

4. Research in regenerative medicine and drug development employing iPS cells and gene-modified non-human primate, Hideyuki Okano, Keio University

SEMINARS OF THE INSTITUTE FOR VIRUS RESEARCH

Twenty-two seminars were held at the Institute for Virus Research under the auspices of the Institute in 2010. Seventeen lectures were from abroad and five others were from Japan.

February 10 Dr.Alain Krol, CNRS, France. “An unanticipated complexity to incorporate selenocysteine into proteins important for health and disease”.

March 9 Dr. Man Lung Yeung, National Institute of Health, U.S.A. “HTLV-1 and small RNAs”.

March 11 Dr. Tadaaki Miyazaki, Hokkaido University, Japan. “ Study of host defence mechanism and pathogenesis in influenza virus infection”.

March 17 Dr. Rod Bremner, Toronto Western Research Institute, U.S.A. “Integrating the cell cycle with neurogenesis”.

March 29 Dr. Yang Shi, Harvard Medical School, U. S. A. “Histone demethylases: mechanism of action and connection to human diseases ”.

April 22 Dr.Yuichiro Justin Suzuki, Georgetown University Medical Center, U.S.A. “Search for therapeutic strategies to treat pulmonary hypertension”.

April 26 Dr. Hiroyuki Niida, Nagoya City University, Japan. “Essential role of Tip60-dependent recruitment of ribonucleotide reductase at DNA damage sites in DNA repair during G1 phase”.

May 14 Dr. Yoichi Kosodo, RIKEN, Japan. “Comprehensive study of the interkinetic nuclear migration of neural progenitor cells; Temporal and spacial analysis of cellular behaviors in the developing tissue ”.

June 9 Dr. Elias Arnér, Karolinska Institutet, Sweden. “Human thioredoxin reductase 1 splice variants regulate cell function through intriguingly complex mechanisms”.

July 16 Dr. Stefano Stifani, Montreal Neurological Institute McGill University, Canada. “ Molecular mechanisms controlling brain development from pluripotent neural stem/progenitor cells”.

August 25 Dr. Ayumu Akahata, National Institutes of Health, U. S. A. “Development of an anti-Chikungunya virus vaccine emloying virus-like particles”.

September 14 Dr. Srinivas S. Rao, National Institutes of Health, U.S.A. “Comparing the efficacy of immunogens and evaluating aerosol delivery of gene-based vaccines against influenza in ferrets, HIV/SIV in monkeys”.

October 8 Dr. Charles R.M.Bangham, Imperial College London, UK. “How does HTLV-1 persist in vivo? ”.

October 29 Dr. Renaud Mahieux, Ecole Normale Supérieure de Lyon, France. “HTLV-1&HTLV-2 similarities and major differences”.

November 5 Dr. Cecilia Cheng-Mayer, Aaron Diamond AIDS Research Center, U. S. A. “Tropism Switch in R5 SHIV infected macaques”.

November 5 Dr. Sarah Bray, University of Cambridge, UK. “Decoding the Notch response”.

November 12 Dr. Junji Takeda, Osaka University, Japan. “Establishment of mutant ES cell bank ready to analyze gene functions and available for many scientists”.

November 15 Dr. Yi Zhang, University of North Carolina, U. S. A. “Could the DNA demethylase please stand up?”.

November 25 Dr. Anna Bigas, PRBB Barcelona, Spain. “Notch signaling in the generation of hematopoietic stem cells”.

December 2 Dr. Yukako Nishimura, National Institutes of Health, U.S.A. “An RNAi screen of microtubule-regulatory proteins identifies MARK2/Par1 as an effector of Rac1 that mediates polarized microtubule growth”.

December 14 Dr. Masafumi Takiguchi, Kumamoto University, Japan. “Evolution of escape mutant HIV from cytotoxic T cells”.

December 16 Dr. Kiyo Sakagami, Jules Stein Eye Institute, UCLA , U.S.A. “Essential roles of PTEN/PI3K signaling in proper retinal network formation”.